The optimised production of 5-HMF and alternative bioproducts from spent coffee grounds in an integrated biorefinery

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Abstract

Spent coffee grounds (SCG) are the organic residue obtained from the coffee brewing process, and are produced in industrial, commercial and residential settings. Approximately 9 million tonnes of SCG is now produced globally, yet is still largely disposed of in landfill or sent to low value food waste processing, such as Anaerobic digestion. However, SCG contain a huge abundance of potentially valuable biomolecules, including fatty acids, proteins and polysaccharides that could be further valorised to produce fuels and chemicals.

To fully exploit the value from the biomass then an integrated biorefinery can be established. This type of biorefinery includes multiple platforms that produce an array of products, maximising the potential value from a feedstock, while reducing the waste produced. This is normally accomplished by fractionating the biomass or reusing the waste streams in subsequent conversion processes to yield increased high-value production in addition to the main product obtained in the biorefinery. As such, integrated biorefineries can normally handle a large variation in the feedstock entering the system.

In this thesis, SCG were evaluated as a potential biorefinery feedstock towards the production of biofuels and higher-value chemicals. Initially, SCG were considered as the only feedstock in a biorefinery configuration aimed at the production of 5-hydroxymethylfurfural (HMF) as the main product. The feedstock was firstly processed in an organosolv fractionation to separate the biomass into an aqueous fraction, a hydrophobic bio-oil and a solid material. It was found that the organic and aqueous fractions were rich in lipids and depolymerised hemicellulose, respectively. These fractions were characterised and presented the potential to be further used in some biorefinery processes. The solid fraction obtained from the organosolv fractionation was rich in cellulose. This fraction was further processed in a combination of enzymatic hydrolysis and enzymatic isomerisation to yield fructose, which was dehydrated to yield HMF.

Following on the success of demonstrating that SCG can effectively be used as a feedstock in a HMF biorefinery, SCG were tested as a potential blending agent with a microalgae strain of *Scenedesmus acutus*. The blends of these two biomasses were tested in a biorefinery designed towards the production of bioethanol, lipids, a biocrude oil and biochar. The configuration of this biorefinery was initiated with an acid pretreatment to depolymerise the biomass macromolecules into fermentable sugars. The produced sugars were then fermented into bioethanol using a strain of *Saccharomyces*

cerevisiae. This fermented broth was then submitted to a lipid extraction followed by an extraction of the produced bioethanol, the presence of which aided in the lipid extraction process. The solid extracted stillage obtained was finally submitted to a hydrothermal liquefaction to produce biocrude and biochar, The use of blends of SCG with microalgae demonstrated higher production of carbohydrates and slightly lower lipids extracted than the microalgae when processed alone in the same biorefinery configuration. This study demonstrated that blends of SCG with microalgae can effectively tackle the seasonality problem associated with microalgae-based biorefineries.

Microalgae production has numerous advantages, including the rapid production that can be carried out in non-arable land. However, this requires a relatively large area to install the microalgae production facility, the technology and investment to do this and a higher value portfolio of products to make it economically viable. An alternative, that is gathering a large amount of research interest recently, is the production of fuels from macroalgae (seaweed) that can take place both in freshwater and saltwater ecosystems. This flexibility presents an opportunity towards this biomass type as there are large areas of saltwater that can be explored in the macroalgae production. However, just like in microalgae, macroalgae also present a seasonality problem. To this end, two strains of macroalgae, *Ulva lactuca* and *Chorda filum*, were blended with SCG and tested in an integrated biorefinery approach designed for the production of HMF. The feedstock was initially submitted to an acid dehydration yielding HMF, which was subsequently extracted. The HMF-free stream was then submitted to a second acid dehydration to produce more HMF, which was also extracted in a downstream process. The resulting solid stream obtained was rich in lipids, lignin, protein and unreacted carbohydrates. This stream was finally processed in a hydrothermal liquefaction to produce biocrude and biochar. The blends of SCG and macroalgae proved to effectively mitigate the availability of macroalgae during low production seasons. In addition the combination of a lipid rich SCG source, coupled with the high C_6 content of macroalgae presented improved conditions towards the production of HMF, with no isomerisation needed to produce the HMF and higher yields of HMF in the combined system for when SCG and microalgae when processed separately.

Ultimately, SCG is a highly suitable feedstock for the production of biofuels and chemicals in an integrated biorefinery concept. The relative lack of supply of SCG, albeit all year-round production, can be mitigated against by blending with alternative feedstocks to produce an array of suitable components including HMF.

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Pereira, Andre Prates, Tao Dong, Eric P. Knoshaug, Nick Nagle, Ryan Spiller, Bonnie Panczak, Christopher J. Chuck, and Philip T. Pienkos. "An alternative biorefinery approach to address microalgal seasonality: blending with spent coffee grounds." Sustainable Energy & Fuels (2020).

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CSCT & US Department of Energy Showcase Symposium (University of Bath, Bath, UK), May 2019, Poster presentation, “Integrated biorefinery to produce value chemicals and fuels from microalgae blended with spent coffee grounds”.

Abbreviations

5-HMF – 5-hydroxymethylfurfural

AFDW – Ash free dry weight

AlkaPolP – Alkaline polyol pulping

CAP – Combined algal processing

CF – *Chorda filum*

$CF_{0.4}$+$SCG_{0.6}$ – Blend composed by 40% of *Chorda filum* and 60% of spent coffee grounds

$CF_{0.6}$+$SCG_{0.4}$ – Blend composed by 60% of *Chorda filum* and 40% of spent coffee grounds

CEF – Cellulose-enriched fraction

DAD – Diode array detector

DCM – Dichloromethane

DDGS – Distiller's dried grains with solubles

DMF – Dimethylfuran

DoE – Department of Energy

DW – Dry weight

FA – Fatty acids

FAME – Fatty acid methyl ester

FDCA – 2,5-Furandicarboxylic acid

FT-IR – Fourier-transform infrared

GC – Gas chromatography

GC-FID – Gas chromatography using a flame ionisation detector

GC-MS – Gas chromatography-mass spectrometry

GGE – Gasoline gallon equivalent

GVL – γ-Valerolactone

HCSD – High carbohydrates *Scenedesmus acutus*

HEF – Hemicellulose-enriched fraction

HHV – Higher heating value

HMF – 5-hydroxymethylfurfural

HPAEC-PAD – High performance anion-exchange chromatography with pulsed amperometric detection

HPLC – High-performance liquid chromatography

HTC – Hydrothermal carbonisation

HTL – Hydrothermal liquefaction

ICP-OES – Inductively coupled plasma atomic emission spectroscopy

IEA – International Energy Agency

LC – Liquid chromatography

LEF – Lignin-enriched fraction

LPG – Liquefied petroleum gas

MAW – Solvent system composed of methyl isobutyl ketone, acetone and water

MEW – Solvent system composed of methyl isobutyl ketone, ethanol and water

MIBK – Methyl isobutyl ketone

MW – Solvent system composed of methyl isobutyl ketone and water

MUFA – Monounsaturated fatty acids

NMR – Nuclear magnetic resonance

NREL – National Renewable Energy Laboratory

PEF – Polyethylene furanoate

PET – Polyethylene terephthalate

SCG – Spent coffee grounds

ssNMR – Solid-state nuclear magnetic resonance

TGA – Thermal gravimetric analysis

TN – Total nitrogen

TOC – Total organic carbon

UK – United Kingdom

UL – *Ulva lactuca*

$UL_{0.4}$+$SCG_{0.6}$ – Blend composed by 40% of *Ulva lactuca* and 60% of spent coffee grounds

$UL_{0.6}$+$SCG_{0.4}$ – Blend composed by 60% of *Ulva lactuca* and 40% of spent coffee grounds

USA – United States of America

USD – United States dollar

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Chapter 1

Literature review

1.1 Introduction

1.1.1 Motivation for biorefinery development

The rapid growth of the global economy has been largely driven by energy dependent industries. Some of these industries, like chemical or fuel manufacture and electricity generation are still heavily reliant on fossil fuel-based feedstocks. However, this exploitation and dependency on fossil-based feedstocks has led to many issues such as price fluctuations, fast consumption of fossil reserves and negative environmental impacts in term of global warming and climate change. Therefore, it is vital that new more sustainable routes to energy and materials manufacture are developed. The transportation sector is predominantly reliant on fossil-based feedstocks, and accounts for up to 25% of all greenhouse gas emissions. In addition to this, the increasing acquisition of cars worldwide accompanied by an increase in the fuel consumption and therefore in the combustion gases released into the atmosphere led petroleum companies to accelerate their fuels production. This implies higher amounts of fossil fuels to be used in refineries which led to a higher exploitation of fossil fuel reservoirs. The current predictions calculate that petroleum will last until the 2050s (1), before extreme price volatility driven by dwindling confidence in reserves becomes the norm. One potential replacement for fuels and chemical are derived from biomass which will inevitably be part of the transition from fossil fuels to a full renewable energy sector.

Biomass composition highly depends on its nature and place of origin. The most commonly biochemical composition of biomass includes carbohydrates, lignin, lipids, proteins and other biomolecules, though this is of course dependent on the biomass type. To this end, biomass can be processed in a combination of chemical and/or biochemical processes to yield value added products similar to a refinery. This has led recently to the concept of integrated biorefinery. Where multiple feedstocks can be processed in combination using multiple production routes, and represents an optimal opportunity to achieve the full valorisation of the biomass using a combination of chemical, thermochemical, biochemical and biological processes to achieve the desired products and by-products. In this concept, the production methods in the biorefinery are organised in such a design to achieve the maximum possible yields of the main bioproducts and to use the biomass to its maximum extent without any or with minimal waste.

Biorefineries can comprise a few or several conversion processes. These correspond to the units where biomass is transformed and/or separated into products or intermediates that can be further upgraded in subsequent conversion units. The conversion processes

used in biorefineries can be grouped by three main types: thermochemical, chemical and biochemical.

There are multiple pathways for biomass to be converted into value added products. The various possible combinations of processes lead to a wide range of possible products (Figure 1-1).

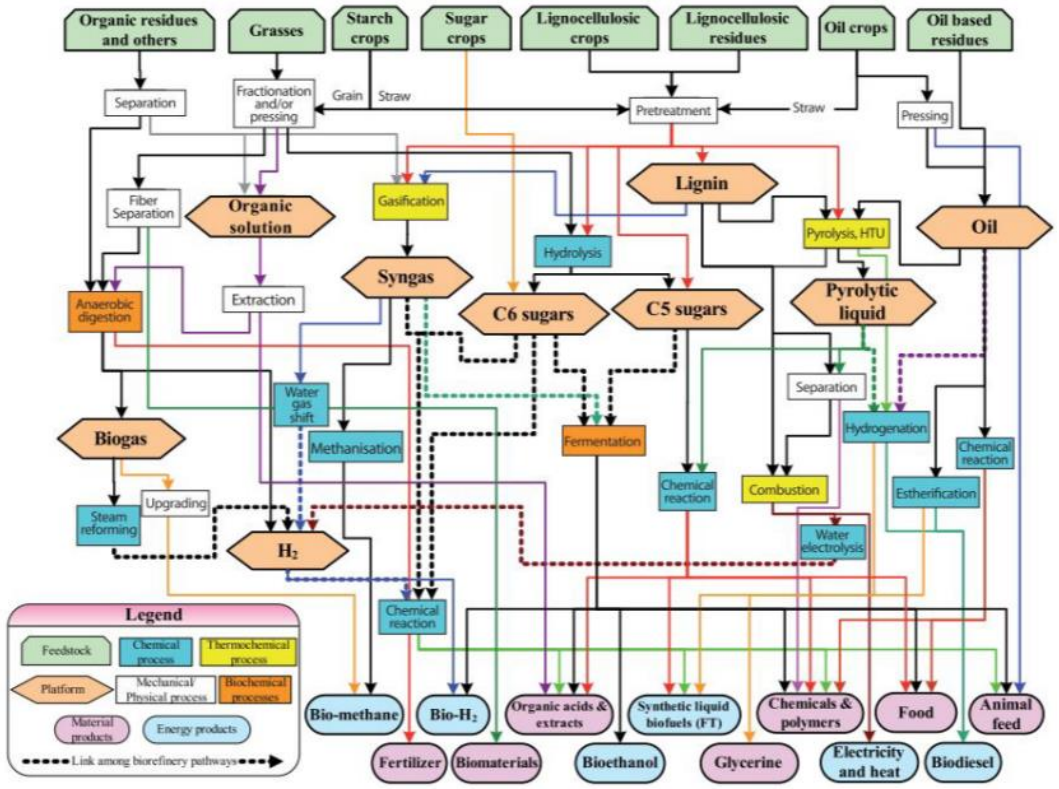

Figure 1-1 – Possible pathways from the different types of biomass into products possible to be obtained in a biorefinery (adapted from de Jong et al (2))

1.2 Pretreatment

Biomass must be pretreated first, to allow further processing. The pretreatment is one of the most important processes in a biorefinery design as it converts the biomass in its most native form to intermediates that can be converted to the final products and by-products in the downstream processes. It is considered a critical operation in a biorefinery as it has a direct influence on the downstream processes results.

There are a large range of suitable biomass pretreatment operations presented across the literature. Most of these focus on the degradation of the raw biomass into oligosaccharides, which can then be further processed into sugars. This is highly dependent on the severity factor that can be defined as the effect of conditions such as the temperature, acidity and duration of the process in the biomass constituents (3). The produced monosaccharides are a suitable platform for fermentation or dehydration into further chemical building blocks. Other pretreatment methods include physical treatment, biological pretreatments and chemical fractionation that focus on dividing the biomass main components (cellulose, hemicellulose and lignin) into different fractions to be upgraded separately in downstream processes. Therefore, the pretreatment process must be carefully planned always taking into consideration the final products desired.

1.2.1 Physical pretreatment

The physical pretreatment of biomass, commonly known as comminution, is a key operation in lignocellulosic biorefineries. Regardless of the biomass type to be used in the biorefinery, this biomass needs to go through a grinding milling process that intends to decrease the biomass into a more digestible size for the following operations. The desired size can be in between the range of micrometres to centimetres, which depends on the downstream processes: under 20 μm for food packaging applications, below 100 μm for combustion and gasification processes, and between 100 and 500 μm for biofuel production (4,5).

1.2.2 Steam pretreatment

The biomass is pretreated with steam (160 °C to 270 °C) and pressures between 0.4 and 4.8 MPa. Such conditions lead to the steam diffusion in the biomass, which condenses into hot water in the biomass microporous (6). This hot water helps in the biomass heating process which releases organic acids from hemicellulose causing a decrease in the pH (7). The release of these acids cause a slightly acidic pH and act as a catalyst in the hydrolysation process of the hemicellulose and degradation of lignin into smaller molecules (8,9). This depolymerisation of hemicellulose and lignin makes the cellulose in the solid phase more accessible to downstream processing. Once the vessel

pressure is released rapidly, a flash evaporation of the hot water inside the biomass microporous occurs, which triggers an explosion of the biomass causing a rupture of its fibres.

Advantages of this process include the lack of chemicals used and low energy requirements, while disadvantages comprise the incomplete depolymerisation of the lignocellulosic structure, the risk of extensive polymerisation of hemicellulose monomers producing inhibitors to the downstream processes and the need to wash and/or neutralise the aqueous fraction obtained.

1.2.3 Liquid hot water pretreatment

This process also known for hydrothermolysis is similar to the steam pretreatment. As the names indicates it uses liquid hot water instead of the steam used in the previous method. Temperatures are usually between 160 and 230 °C. Just like in the previous process, the hemicellulose is hydrolysed into the aqueous phase and the lignin is decomposed into smaller molecules by the same principle, leaving the cellulose in the solid phase.

Advantages of this process include the low energy requirements due to the low temperatures used in the process. Such temperatures decrease the probability of inhibitor formation, which also acts as a double advantage as it disregards the need for a washing/neutralisation step of the aqueous stream. The biggest disadvantage of this process is the lower concentration of the mono and oligosaccharides in the aqueous phase due to the higher amounts of water involved in the process. Further disadvantage is the possibility of an extensive hydrolysis degrading the monosaccharides into aldehydes. Such aldehydes can act as inhibitors in downstream processes such as fermentation. However, this can be avoided by controlling the medium pH in the range between 4 and 7.

1.2.4 Acid pretreatment

This method has received broad attention and has been used widely in research due to its hydrolysis effect on the biomass. Sulfuric acid is commonly chosen as acid in concentration lower than 4% w/w. Reaction temperatures range from 140 to 215 °C. Residence times vary between seconds to almost an hour. Previous research has demonstrated that a two-step acid pretreatment can be carried out to improve sugar recovery (10). The concept of this process is relatively similar to the one in liquid hot water pretreatment. However, the acid is added to the solution, which induces further release of acids, resulting in a higher severity factor for this pretreatment. The severity factor of the pretreatment should take into account the downstream processes and the

desired products. While inhibitors such as furfural and 5-hydroxymethylfurfural might not be desirable in the fermentation process, these aldehydes can be valuable intermediates in the upgrading to high value chemicals and biofuels.

The high conversion rate of biomass into oligo and monosaccharides is the main advantage of this process. On the other hand, the extensive hydrolysis may be seen as a disadvantage if fermentation is the subsequent process. The use of acid can act as a double disadvantage as it makes the process more expensive (higher impact on large scale) and requires a more costly reactor construction due to its corrosive effect. On another hand, some of the products of an extensive hydrolysis, such as furfural and 5-hydroxymethylfurfural, are high value products that can be recovered after the pretreatment and add value to the biorefinery.

1.2.5 Carbon dioxide explosion

This method uses supercritical carbon dioxide to improve the depolymerisation of the biomass. The gas stream is fed to the system under high pressures (about 70 to 270 atm) and the reactor is heated until the set temperature (up to 200 °C) at which it is held for the duration of the process (3). The CO_2 dissolves in water and forms carbonic acid, lowering the pH of the solution which helps in the hydrolysis process of biomass. The high pressures lead to the diffusion of the liquid phase and into the biomass microporous and increases the depolymerisation yields. Once the high pressure is released the vaporisation of the liquid phase inside the microporous occurs, inducing the explosion of the biomass. Advantages to the process are the high solids capacity of the process and the low prices of the solvents (water and CO_2), however the high pressures used require high cost equipment.

1.2.6 Organosolv Fractionation

There is a growing interest in organosolv fractionation for biorefineries as it offers a different type of pretreatment when compared to the previously studied pretreatments. This operation not only depolymerises the biomass into its oligomers and monomers but also allows the separation of the biomass into three separate fractions of its main constituents: cellulose, hemicellulose and lignin enriched fractions. The reaction is carried out at temperatures between 100 and 250 °C, which depends mostly on the use of acid. Higher temperatures do not require any acid as such temperatures are enough to depolymerise the biomass and releasing organic acids from the process. However, those temperatures are energy consuming and the addition of acid that works as a catalyst decreases the temperatures to start the reaction. In fact, it was found that the addition of mineral acids such as sulfuric acid, hydrochloric acid or phosphoric acid

increases the level of delignification and xylan depolymerisation of the pretreatment (11–13). The name organosolv fractionation origins from the use of a solvent system composed of an organic solvent and water (14–16). This organic solvent are typical methanol, ethanol or higher molecular weight alcohols and/or ketones (11).

A variant of the organosolv fractionation, termed clean fractionation, has been drawing the attention of the researchers in the last years (17,18). This method uses a ternary system of solvents (water, ethanol and methyl isobutyl ketone) to isolate a relatively rich cellulose fraction into the solid phase, while the hemicellulose is separated into the aqueous fraction and the lignin in the organic fraction. Since this point, acetone has replaced ethanol as this solvent has a lower boiling point which makes its recycling less energy demanding, an improved separation of the organic and aqueous fractions without the use of any salts (19). This approach has been of relative interest in the last years as it has been widely used in the literature (20–28).

Advantages of this operation include the easy recycling of solvents and separation of biomass main constituents into different streams while also depolymerising the biomass. Disadvantages are the need for washing the solids with the reaction solvent followed by a water wash and the energy associated with the recycle of the solvents.

1.2.7 Biological pre-treatment

Unlike the alternative pretreatment operations, the biological pretreatment does not require high temperatures. Most biological pre-treatments use fungi to depolymerise the biomass. White-rot, soft-rot and brown-rot fungi are the three main types of fungi studied in the lignocellulosic biomass degradation. Processes based around white-rot fungi appear to offer the best results as it acts primarily in the delignification of the biomass which then results in better saccharification yields (3,29,30). This is then followed by soft-rot fungi and lasted by brown-rot fungi that focuses on the depolymerisation of cellulose that is “protected/shielded” by the lignocellulosic structure of the biomass and therefore leads to lower saccharification yields. This operation is mostly used as a pretreatment step previous to the fermentation of the resulting broth towards the production of bioethanol and further upgrading of the remaining streams to biogas, methane and transesterification of the lipids to biodiesel (30).

A few of the biggest downsides with this technology are the extremely long residence times of the biomass (10 to 14 days), which are simply impractical compared to the alternatives, and the large area of land required to install the equipment necessary for this operation.

1.3 Biorefinery conversion platforms

1.3.1 Thermochemical conversion processes

Thermochemical processes use elevated temperatures to promote the depolymerisation and deconstruction of the biomass. The most commonly used thermochemical processes include combustion, pyrolysis, gasification and liquefaction.

Combustion is the simplest energy conversion process in which biomass is subjected to. In 2004 the energy produced from biomass combustion represented around 97% of the total energy produced by biomass (31). Although this process is still commonly seen across several industries, biomass also represents a wealth of functionality which make it ideal for the production of more valuable chemical precursors and liquid fuels.

The most researched conversion technique is arguably pyrolysis. Pyrolysis is undertaken between approximately 350 °C and 650 °C, on dry biomass feeds in the absence of oxygen. Pyrolysis can be divided into three different types: slow, fast and flash pyrolysis.

Slow pyrolysis is characterised by a long residence time - it can last hours or even days – at a slow heating rate (lower than 10 °C per minute). The final product of this process is targeted to be an energy dense carbonous char (usually known by charcoal) aimed for heating purposes. With fast pyrolysis the heating rate is much higher (approximately 1000 °C per second) and the residence time is much shorter (in the order of seconds). The high temperature rates cause the drying and volatilisation steps to happen nearly instantaneously resulting in a gas phase that is separated from the solid phase by filters or cyclones. Another characteristic of the fast pyrolysis is the fast quenching applied to the gas phase resulting in a bio-oil (maximum yield approximately 70% w/w of the biomass). Apart from the bio-oil, also char is produced from this process.

While fast pyrolysis is designed for the production of bio-oil, flash pyrolysis is more focused on charcoal. Unlike the previous pyrolysis processes, flash pyrolysis occurs at higher pressures than the atmospheric one (between 2 and 25 atm). Compressed air is injected in a high-pressure chamber where electric heaters initiate the reaction at the bottom of the reactor. The process lasts about 30 to 45 minutes. The gas phase can be further used in the production of heat and power.

At higher temperatures, gasification process occurs (750 °C to 1500 °C) and can be carried under pressure or atmospheric pressure. The process is relatively similar to combustion, however the lower concentrations of oxygen in the chamber (usually in stoichiometric ratios) and the low residence time (seconds to few minutes) do not allow for a complete combustion. In fact, instead of yielding syngas, containing hydrogen and

carbon monoxide as its main constituents as well as methane, short hydrocarbons and carbon dioxide. The main applications of syngas include its conversion to methane as an alternative to natural gas, methanol and hydrocarbons through Fischer-Tropsch synthesis.

In addition to syngas, tar is also obtained from the process as a by-product. Ideally, it should be char but the short residence time in the gasification chamber is not enough to reach thermodynamic equilibrium, leading to viscous tars that may present issues downstream to this process.

The gasification process is often found alongside a Fischer-Tropsch synthesis as the latter uses the downstream main product of the first one, syngas. Carbon monoxide and hydrogen are submitted to a high pressure environment at moderate temperatures in the range from 200 °C to 350 °C depending on the final product choice (32). On one hand, the lower temperatures process (200 °C to 240 °C) is often catalysed by a cobalt or iron-based catalyst is designed to produce paraffin waxes. On the other hand, the process using higher temperatures (300 °C to 350 °C), commonly using an iron-based catalyst, yields short chain hydrocarbons and gasoline (32).

Such reactions often happen in a fluidised catalyst bed reactor as this design allows for fast cooling of the catalyst that would otherwise overheating which could lead to catalyst deactivation and an undesired production of methane (32). Challenges to this process include the inlet hydrogen:carbon monoxide ratio (2:1) or the fouling on catalyst, which can be avoided by removing syngas contaminants in a gas conditioning process after gasification. However, such process is usually expensive (33,34).

Biomass can also be thermally processed in water. This is termed hydrothermal carbonisation (HTC), to produce a solid fuel, or hydrothermal liquefaction (HTL) to mainly produce bio-crudes (35). Hydrothermal carbonisation occurs in the presence of water at temperatures between 180 and 250 °C at moderate to high pressures (25 atm) to ensure the water remains in its liquid state. The reaction can last from minutes to hours, even though some authors conjectured most of the coal is produced in the first 20 minutes (36). Whereas hydrothermal liquefaction is undertaken at 250 – 370 °C over rapid time scales (37). This results in a biocrude product, as well as a aqueous phase containing nutrients and a char. Sevilla and Fuertes suggest that the initial step in the hydrothermal process is the hydrolysis of cellulose and hemicellulose into its oligomers, due to the presence of water (38). This is followed by further hydrolysis into monomers, organic acids, furfural, 5-hydroxymethylfurfural and aldehydes which undergo further

dehydration and decarboxylation to liquid intermediates before the polymerisation to either char or bio-crude takes place (38).

For HTC the main product from this process is a coal with low moisture content and lightly hydrophobic making it good for storage. One of the great advantages of this process is its high efficiency as most of the carbon from the feedstock is converted to coal with little production of carbon dioxide and methane (39). The HTL process is similarly efficient and results in a far more stable bio-crude than pyrolysis. Further advantages of both processes include the fact that this is an exothermic process and the use of the resulting water as fertilizer due to the excess nutrients dissolved in it.

One of the main attractions of HTL is the high heating values of the bio-oil produced (40). Also, the moisture levels of the biocrude are also lower when compared to the one produced by pyrolysis, which not only has a positive impact on its storage but also a reduction of fixed and operating costs of the handling and storage equipment (41). However, the higher pressures registered in HTL require more robust reactors that intrinsically are more costly.

In addition to the biocrude produced in HTL, biogas and biochar are the by-products in this process. Considerable amounts of aqueous phase are also generated in this process. Instead of spending energy and funds on the treatment of this stream previous to discharging, higher value applications for this phase include recycling to a new HTL reaction, as a growth media for algae, its use in supercritical water gasification or anaerobic digestion. It has been demonstrated that the recycling of the aqueous phase can lead to higher biocrude yields and improved high heating values in addition to a reduction in freshwater consumption (42,43). Also, the nutrients in the aqueous phase (mostly phosphorous and nitrogen) make this stream a suitable growth media for microalgae even though this stream can carry some inhibitors such as phenols and metals which would require a dilution of such phase (44,45). The aqueous phase can be used in supercritical water gasification to yield a gas rich in hydrogen (46). Finally, anaerobic digestion can be used in the aqueous phase to convert the organic compounds (that were not degraded during the HTL process) into biogas, however the low efficiency registered is still a challenge (47,48).

1.3.2 Biological conversion platform

Biological conversion operations deal with the transformation of raw feedstocks and intermediate materials by using living whole cell microorganisms or enzymes derived from biological species to perform these operations.

1.3.2.1 Enzymatic hydrolysis

In an integrated biorefinery context, the enzymatic hydrolysis is commonly applied after the pretreatment and before fermentation. This is because the slurry obtained from pretreatment can have relatively high concentration of oligomers and polymers such as cellulose. In most cases, these molecules cannot be consumed by the fermenting agents. Therefore, a saccharification of such molecules is required.

The most commonly used enzymatic hydrolysis is the hydrolysis of cellulose and cellobiose using cellulases. The cellulase is adsorbed onto the cellulose surface, which performs the depolymerisation into glucose and is then followed by a desorption of the cellulase. The cellulase is then adsorbed in another cellulose molecule to repeat the cycle. This process is carried out under mild conditions, usually at temperatures between 40 and 50 °C and pH in the range from 4 to 5 (49).

Enzymatic hydrolysis presents some advantages when compared to acid hydrolysis. Enzymatic hydrolysis is carried out at lower temperatures and more neutral pH values, achieving higher yields. In fact, according to Ogier et al, an cellulose hydrolysis yield close to 100% yield can be achieved using enzymatic hydrolysis (50). Enzymatic hydrolysis does not produce any inhibitory compound, contrary to what can happen in the acid hydrolysis (51,52). However, enzymatic hydrolysis has some drawbacks such as the long residence time required, up to several days, contrary to the few minutes required in the acid hydrolysis. Higher cost of enzymes compared to acids such as sulphuric acid and the inhibition of further hydrolysis caused by the produced sugars represent other drawbacks.

1.3.2.2 Fermentation

Fermentation is commonly used to mean a process using a life cellular organism converting a carbon source into a range of useful products. This is one of the most used operations in biorefinery studies with over 60 industrial conversions now developed (53). The conditions (temperature, pH and aerobic/anaerobic atmosphere) and the product obtained depend on the microbial agent used. In fact, there are five main types of fermentation processes: focused on the production of microbial biomass, microbial enzymes, microbial metabolites, recombinant products and focused on the modification of a compound added to the fermentation process (also known by transformation process) (54). The production of microbial biomass focuses mainly on the production of yeast for baking purposes and the production of microbial cells for human and animal feed in the form of protein. The production of enzymes, as the name says, focuses on the production of these biological entities to be used in a wide range of applications,

going from baking, brewing, soft drinks, dairy, laundry to pharmaceutical products. Microbial metabolites fermentation aims to yield products of considerable economic importance such as alcohols, organic acids (citric acid, butyric acid or glutamic acid), nucleotides or vitamins. Recombinant products fermentations use genetically engineered organisms to yield important peptides such as insulin, interferon or bovine somatostatin. The last fermentation type uses microbial cells to perform a transformation process on compounds to higher value related products. Dehydration, isomerisation, dehydrogenation, decarboxylation, oxidation, amination or deamination are some of the conversion processes in this type of fermentation.

The most commonly type of fermentation found in biorefinery studies is the microbial metabolites fermentation. The production of bioethanol is one of the most used operations. Bioethanol is a more sustainable replacement for gasoline in the transport sector. To perform this process, the biomass has to go through a pretreatment step to depolymerise it into fermentable sugars. These fermentable sugars are then consumed by the yeast that produces energy, carbon dioxide and ethanol, a process can be described by the Krebs cycle. Other fermentations commonly found in the literature include the production of butyric acid, butanol or biohydrogen (55).

1.3.2.3 Anaerobic digestion

This process was initially developed to deal with the sewage sludge in municipal treatment plants. In these plants the sludge is put in contact with microorganisms in the absence of air, converting the organic waste to carbon dioxide and methane (mixture of gases commonly known as biogas). However, the current incentive towards the replacement of fossil fuels with the production of fuels from renewable sources has drawn an increased attention to this process (56). The wide range of biomass and biowaste feedstocks that can be used in this process are also an advantage towards its use. The concept behind this operation is based on methanogenesis that consists on the organic matter molecules being broken down by the microorganisms into the carbon dioxide and methane. Biogas composition is usually found to be 60% of methane and 40% of carbon dioxide (57). After the anaerobic digestion process the methane is commonly separated from the carbon dioxide and the other vestigial gases, so that it can be used as an energy source.

Compared to the aerobic digestion, the anaerobic process has the advantage that the absence of oxygen does not allow a fast growth of the microorganisms, which by its turn, leads to a higher conversion of the waste/biomass into biogas (58). When compared to thermochemical gasification (other process that converts biomass to biogas), anaerobic

digestion has the advantage that biomass does not need to be dried previous to this process. The energy savings from the non-drying of biomass are of particular interest mainly for biomass feedstocks with high moisture content (57).

1.3.3 Chemical conversion platform

1.3.3.1 Hydrolysis and dehydration

Hydrolysis is a common chemical process used to break down biomass macromolecules in the presence of water into its monomers. The most common used variant of hydrolysis is the acid catalysed hydrolysis. This process is usually carried at medium temperature in the range of 100 and 150 °C and the main products aimed in the biorefinery literature include 5-hydroxymethylfurfural (HMF), furfural, levulinic and formic acid (59–61). Furfural has a 5-carbon structure which makes it possible to be obtained from C5 sugars (pentoses) present in the hemicellulose, while HMF with a 6 carbons structure is derived from C6 sugars (hexoses such as mannose and glucose). Levulinic and formic acid are products of an extended hydrolysis process which leads to a degradation of the monomeric sugars. These products have a high importance in the market as it was demonstrated when the United States Department of Energy (DoE) included HMF and levulinic acid in the top 12 bio-based platform chemicals (62).

Contrary to the hydrolysis, the dehydration process consists on the loss of a molecule of water from one or two molecules. The most common dehydration found in literature is the Fischer esterification which uses an alcohol and a carboxylic acid to yield an ether. This process is usually catalysed by an acid (commonly sulfuric acid).

1.3.3.2 Transesterification

The transesterification process is a classic organic reaction with many industrial applications: from the polymerisation of lactones to the process of curing alkyd resins and the production of polyester. However, this process has received an increased interest in the biofuels field in the recent years as it is responsible for the production of biodiesel from lipids. The reaction takes place when a carboxyl acid is put in contact with an alcohol, producing an ester as the name indicates. This reaction occurs naturally as it is an equilibrium process. However, research have shown that this reaction can be catalysed by an acid or base (63,64).

1.4 Major industrially produced fuel products from biorefineries

From these platforms a range of products can be produced. To this end, this section discusses the most commonly produced biofuels and their precursors obtained in an integrated biorefinery context.

1.4.1 Heat and power

Heat and power are amongst the most conventional forms of energy obtained from biomass. Power generation starts with the combustion of the biomass in a combustion chamber where a stream of water circulating in a closed system is heated and converted to steam due to the high temperatures registered in the chamber. This steam passes through a turbine that induces the movement of an electrical generator that converts the mechanical energy into electrical power. When the heat is also collected from the steam exiting the turbine and going through a condensation process, the unit is called combined heat and power plant instead of just power plant. Currently, approximately two fifths of the biomass power units in Europe are producing both power and heat (65).

The International Energy Agency reported that 2019 had an increase of 5% in power production from biomass relatively to the previous year (66). Such results fall under the 6% goal to achieve the Sustainable Development Scenario in 2030. However, a growth in this percentage is expected in coming years with increased production of heat and power from biomass and waste co-generation plants. In fact, the generation of power from municipal waste is increasing its interest in China (66).

1.4.2 Gaseous bioproducts

1.4.2.1 Biogas

This product is mainly composed of carbon dioxide and methane usually in a ratio of 2:3. This gas is obtained from the anaerobic digestion of organic matter. One of the advantages to the use of this process and the production of biogas is the flexibility on the choice of feedstock: the microorganisms responsible for this reaction degrade the organic matter from the various biomass generation types to municipal waste (67). Energy wise, biogas has a much lower calorific value (23.1 MJ/m^3) when compared to pure methane (39.8 MJ/m^3), which by its turn has a similar calorific value to natural gas (38.7 MJ/m^3) (68). Therefore, it is common to refine biogas after the anaerobic digestion. This process consists on the removal of carbon dioxide, nitrogen, oxygen, hydrogen, moisture and other trace gases. The resulting gas from the refining process is known by biomethane or renewable natural gas. In fact, if the refining process is thorough enough and the biomethane fulfils the natural gas industry standards, it can be used as a replacement for natural gas in the heat and power industry (69). Additional applications

include its use in the transportation sector, lighting, cooking and further upgrading to chemicals like the Fischer-Tropsch synthesis (67).

Some of the biomethane advantages regarding its use in the transportation sector include its low requirements of air to be combusted and at the same time a high auto ignition resistance (70). However, there are still some challenges regarding the use of gaseous fuels in the transportation sector. The gas has to be stored under high pressure which makes an expansion required for its use. This expansion can cause some alteration in the fuel properties such as density and temperature (68). Also, the presence of carbon dioxide in the biomethane gas can be prejudicial as it leads to an increase in the emissions of carbon monoxide and the total hydrocarbon content. Such properties cause an incomplete combustion and may lead to loss of brake power (71,72).

1.4.2.2 Bio hydrogen

Biohydrogen is an alternative source of hydrogen to steam reforming of fossil fuels (73,74). The interest on hydrogen has increased in the recent years due to its high energy content (122 MJ/kg) and the ability for clean burning (75). Even though hydrogen can be obtained through the gasification of biomass, this process also produces carbon dioxide which would require a refining of the produced gas to remove the carbon dioxide downstream to the gasification process. Such approach leads to increased costs. More recent approaches include the use of water electrolysis, dark or photo fermentation methods. The dark fermentation process has the advantage that it can use a wide range of lignocellulosic wastes as feedstock (76). In this process, the organic matter is digested by anaerobic bacteria under dark conditions, therefore the name dark fermentation.

The advantages of hydrogen as an energy vector include the high energy content, the feedstocks used in its production like water through electrolysis or a wide range of biomass types going from lignocellulosic biomass, to waste biomass or organic municipal waste can be used in dark fermentation (77). Still, one of the main advantages of hydrogen when compared to other energy carriers is that the oxidation of this fuel only generates water and there is no generation of greenhouse gases.

However, there are still some challenges to be dealt with when considering hydrogen as a transportation fuel. These challenges include safe storage and usage (due to the pressures required for is storage and the expansion previous to its use) and how the distribution network would be (this includes type of distribution, centralised or decentralised production, how the distribution would occur during the transition period from fossil fuels to hydrogen, etc).

1.4.3 Liquid biofuels and related chemicals

1.4.3.1 Bioethanol

Bioethanol is one of the most commonly obtained product in biorefinery literature. Ethanol is a colourless liquid with a high octane number which makes it a good feedstock to blend with low octane gasolines. The higher oxygen content in bioethanol makes its combustion cleaner when compared to petrol. However, the bioethanol lower energy content is a drawback (around 68% lower) (78).

The wide range of biomass types (first generation biomass, to second generation biomass and municipal waste) that can be used in the production of bioethanol is also a big advantage of the production of this biofuel. The microorganisms responsible for the fermentation to ethanol, consume sugars, mostly monosaccharides and disaccharides. Therefore, to achieve the highest possible fermentation yield, it is common to observe a pretreatment step previous to the fermentation. This pretreatment depolymerises the high complexity of the biomass macromolecules (cellulose and hemicellulose) into fermentable sugars to be used by the fermentation microorganisms.

The production of bioethanol using yeast has been demonstrated to achieve high ethanol yields, high productivity and high ethanol concentration in solution before it is considered toxic to the yeast growth (79). *Saccharomyces cerevisiae* is the most commonly used yeast strain in the production of bioethanol industrially due to its large range of pH tolerance, easy availability and the low cost associated to the process (80). However, one of the challenges with this strain is the fact it can only ferment hexoses and not pentoses. Still, there are some genetically modified strains that can ferment pentoses which improves the ethanol yield (81,82).

Ethanol is seen as one of the possible biofuels to be used during the transition period to a more sustainable bioeconomy. In fact, the most common application for bioethanol at a global scale is in fuel blending in the transportation sector. This includes ethanol blends of 5 and 10 (E5 and E10, respectively) in petrol. These blends can be used on most of the vehicles built after the year 2001 without modification (83). Moving beyond the E10 includes E65 and E85, however these blends can only be used in a special type of vehicles known by Flex Fuel Vehicles that can run any blend between the range of E65 to E85.

In 2019 approximately 132 billion of litres of bioethanol were produced worldwide, registering an increase of 43% in the last 10 years. These numbers help to show the market increase on this product, which looks to be a trend as bioethanol gradually replaces fossil fuels in the transportation market.

1.4.3.2 Biomethanol

When compared to bioethanol, biomethanol has a higher energy yield (84). The main reason that bioethanol is produced in a large scale is related to the compatibility of the gasoline and bioethanol blends. However, biomethanol has a higher carbon dioxide mitigation potential, which can turn to be a powerful reason to increase its productivity worldwide (85). Biomethanol further advantages include its simple storage at room temperature due to the fact it is a liquid and its high volumetric energy density.

The combination of methanol worldwide plants accounts to a total of 110 million metric tons capacity. However, these are mostly fossil fuel based, and a change to renewable methanol is needed. Biomethanol can be obtained using a wide variety of biomass sources through thermochemical or biological processes. Gasification, pyrolysis and liquefaction are the thermochemical processes used to obtain methanol. These processes yield syngas which is further processed through a Fischer-Tropsch synthesis to produce methanol (86,87). Fast and flash pyrolysis are the most used pyrolysis types in the production of liquid biofuels, mainly from pectin and uronic acid biomass feedstocks (86,87). However, the thermochemical processes have been widely studied and are considered economically unappealing (86). Biochemical processes are an alternative as methanol can be produced by anaerobic digestion. This process yields methane and ammonia that are used for methanol production by methanotrophic and oxidising bacteria, respectively. However, this process is still at its early stage and further research is needed.

The most used application for biomethanol is in the production of formaldehyde further used in coatings and plastics. Further application include the transportation sector, one of the main drivers towards its 6% production increase in 2018 (88).

1.4.3.3 Bio-oil

Bio-oil is commonly associated to the pyrolysis oil, produced from pyrolysis, however other conversion methods such as liquefaction also yield a bio-oil. In any of the cases, bio-oil main constituents are obtained through the cracking of lignin present in biomass. Main constituents include polyphenols and smaller percentages of methylphenols, xylenols and eugenol (89). Bio-oil usual calorific content falls between 16 and 20 MJ/kg. Such values are relatively smaller than other biofuels such as bioethanol due to their content on oxygenated compounds (90). Aside from the oxygenated compounds in bio-oil, other problem is the presence of water which leads to lower calorific contents and lower storage periods. A solution to this problem includes the catalytic pyrolysis of biomass which yields a bio-oil with increased calorific values and higher stability (91).

Alternatively, the bio-oil obtained from HTL reaction is submitted to a phase separation, removing most of the water present in solution.

One of the applications to bio-oil is its use as fuel in a boiler. Further applications with increased value, include bio-oil upgrading to biofuel through hydrodeoxygenation, conversion to high value chemicals by separation of bio-oil constituents, or the production of carbon materials through polymerization. The hydrodeoxygenation on bio-oil leads to a biofuel with increased calorific content (can go up to 42-45 MJ/kg) due to the lower oxygen content and consequently water content in the fuel (92). Separation methods on bio-oil to produce chemicals include distillation (to yield acetol, acetic and formic acid) and extraction by solvent (92). The extraction by solvent process yields an aqueous phase and an organic phase. The organic phase is mostly composed of monophenols to be used in the production of adhesives and resins while the aqueous phase can be used to produce hydrogen, alcohols and bio-gasoline.

1.4.3.4 Biodiesel

It was estimated that the worldwide production of biodiesel in 2019 surpassed 40 billion litres, with Indonesia, USA and Brazil as the main producers (7.9, 6.5 and 5.9 billion litres respectively). Reports show that the trend in the production of biodiesel is set to increase in the coming years, as biodiesel gets used in the transportation sector in fuel blends with diesel (93,94).

Typical biodiesel molecules are fatty acid esters, fatty acid methyl ester being the most common. These molecules are produced by the transesterification process of fats and oils (triglycerides) in the presence of an alcohol. The most used alcohol across the literature is methanol. Alcohols with lower water content are preferred as the presence of water in the reaction leads to the hydrolysis of the triglycerides producing soap, which results in lower biodiesel yields. This is the case with lower chain alcohols such as methanol (high hygroscopicity). Nonetheless, methanol is still the most used alcohol due to the steric hindrance effect (95,96). Previous studies demonstrate that ethanol can also be used due to its low price, the fact that it can be produced entirely from renewable sources and lower toxicity (97).

The most important properties when producing biodiesel include the presence of impurities, low-temperature operability, kinematic viscosity, exhaust emissions, cetane number, energy content, oxidative and storage stability. Low temperature operability include measurements such as cloud point, pour point, cold filter plugging point and low temperature flow test (98). Cloud point corresponds to the temperature at which the formation of crystals with diameter higher than 0.5 μm occurs. The pour point

corresponds to the lowest temperature at which the fuel pours. The cold filter plugging point is related to the lowest temperature at which a certain volume of biodiesel can flow through a previously defined filter size under a certain time limit. A common calorific value for biodiesel is around 37.6 MJ/kg (99).

The other product from transesterification is glycerol that can be further used in the production of high value chemicals. These includes the production of hydrogen, methanol, pharmaceuticals, cosmetics, lubricants and food additives. However, the glycerol obtained from the transesterification process needs to be submitted to a combination of purification steps due to the requirements of downstream industries.

1.4.3.5 Synthetic diesel

So called 'synthetic diesel' has gained increased attention in the last years. This is partly due to the potential of renewable electricity to be used to reduce carbon dioxide to more reactive precursors which can be used to produce synthetic diesel. One of the problems with renewable electricity is its storage and losses during long storage periods. However, using the excess electricity to produce chemicals has been raised as a solution to this problem. In this context, renewable electricity that exceeds the demand can be used to reduce carbon dioxide and produce hydrogen through the electrolysis of water. This hydrogen is submitted to a Fischer-Tropsch diesel reaction yielding synthetic diesel as the main product. However, one of the challenges to this route includes the location of a reliable carbon dioxide source near the synthetic diesel production facility. Samavati et al. suggested an integrated facility with gasification process able to produce the carbon dioxide required in the Fischer-Tropsch reaction (100). Another challenge is if synthetic diesel is to be used as a fuel continuously throughout the year its supply needs to be reliable and the intermittency of renewable electricity might turn to be a problem. Other non-renewable sources of synthetic fuel production include natural gas or the gasification of coal to feed the Fischer-Tropsch process.

The main application for synthetic diesel is in the transportation sector where it can be used neat or blended with conventional diesel. One of the advantages with synthetic diesel is its relatively similar properties when compared to conventional diesel. This has a positive impact due to its compatibility with existing engines and therefore there is no need for modifications. Furthermore, the relatively low concentration of sulphur in this fuel is a positive aspect. Finally, the use of synthetic diesel is considered to recycle carbon dioxide as it is firstly used in the production of the diesel by Fischer-Tropsch and then produced in the diesel consumption, though the overall energy and carbon balance is strongly influenced by the source of the energy used to produce the fuels.

1.4.3.6 Dimethyl ether

Bioderived dimethyl ether is seen as an alternative fuel able to replace fossil fuels in the automotive industry due to its compatibility with diesel engines. This is related to its low self-ignition temperature and cetane number. Ether has similar physical properties to liquefied petroleum gas (LPG), it is immiscible with water and high purity levels makes it an attractive alternative to fossil fuels (101). The similar properties, when compared to LPG, also allow it to be used in similar supply chain infrastructures (102).

Dimethyl ether can be produced using different approaches. It can be obtained by the catalytic dehydration process of methanol. Considering that this methanol is obtained from sustainable sources (e.g. anaerobic digestion of biomass) the fuel can be defined as biodimethyl ether. However, this approach can lead to low conversion efficiencies on methanol synthesis due to thermodynamic limitations (103). As so, recent research suggested an alternative route combining the methanol synthesis and dehydration processes in one single step making it an one-step process for biodimethyl ether production (104).

Other important application of dimethyl ether consists on the manufacture of aerosols in replacement of banned chlorofluorocarbons due to their harmful properties on the ozone layer. Further applications include the production of chemicals such as acetic anhydride, methyl acetate, ethylene or propylene (101).

1.4.3.7 Furfural

Furfural was considered one of the twelve most value-added products by the United States Department of Energy (62). In 2019 the furfural market was estimated to be approximately 815 million USD and is expected to have a growth of around 35% until the year of 2027 (105). Furfural is a precursor that can be used in the production of a wide variety of fuels and chemicals in industries such as plastics, pharmaceutical and agrochemical. It consists of a furan ring with a formyl group on position two of the ring. It can be obtained from a wide variety of feedstocks but there is a particular one that has gained increased attention in the recent years which is hemicellulose from lignocellulosic biomass. As opposed to cellulose, which is a polysaccharide exclusively composed by glucose, hemicellulose is an heteropolymer and can have a very distinct monomeric composition depending on the nature of the biomass.

Hemicellulose is rich in the C5 monomers that undergo acid catalysed dehydration to furfural, and arabinose and xylose are predominantly used. Different biomass feedstocks such as sugarcane bagasse, corn stover, birch wood, corncob, rice husk or wheat straw

have been demonstrated to be efficient feedstocks used in the production of furfural (106–109).

Two different approaches are used industrially, the first approach is a two-step method which consists firstly of a two-phase system (aqueous and organic) to separated hemicellulose from the other biomass components (process previously reviewed as fractionation of biomass). The second step is a biphasic system where the furfural is produced in the aqueous phase and transferred to the organic phase, where it can be isolated after the reaction (110). The second approach consists of an acid catalysed single-step method which converts the hemicellulose into furfural.

Furfural has a wide range of applications. These include the downstream upgrading to an extensive list of chemicals and fuels in industries such as refineries, pharmaceutical, resins and agrochemical. Some of these chemicals include furan, furfural resins, tetrahydrofurfuryl alcohol, methyl tetrahydrofuran, methylfuran, furoic acid, succinic acid and maleic acid. Examples of fuels that can be obtained from furfural comprise furfuryl acetate, levulinic acid and valerolactones derived fuels, bicyclopentane and furfuryl alcohol which can be further upgraded to diesel and kerosene.

1.4.3.8 5-hydroxymethylfurfural

5-hydroxymethylfurfural (HMF) is formed from the acid catalysed breakdown of C6 sugars such as glucose, it has a melting point of 31.5-35 °C. HMF is highly reactive and the presence of impurities accelerate its polymerisation into dimers and oligomers (111,112).

The molecular arrangement of the HMF consists of a carboxyl and a hydroxyl group attached to a furanose ring. This is important as the HMF is mainly obtained by the dehydration of certain sugars (113). However, studies have shown that higher yields of HMF production are achieved when sugars with a 5-atom ring (furanose) are used as feedstock (114,115). For that reason, most of the biomass feedstocks that yield glucose from its depolymerisation, have to be submitted to an isomerisation process to fructose. This can also play an important role when choosing the ideal feedstock for HMF production in a biorefinery.

HMF is a platform chemical from the furan group as it is considered a valuable precursor in a wide range of industries such as the petrochemical, pharmaceutical, resins, solvents and polymer industry. This compound has been gained rising interest in the last years due to its conversion into 2,5-dimethylfural, one of the most valuable biofuel candidates in part due to its high energy density, which is considerably higher than the energy density of bioethanol and comparable to gasoline (116,117). Other derivate that can be

obtained from HMF is 2,5-furandicarboxylic acid. This compound is a valuable building block in the polymer industry, even more when there is the possibility to produce it from renewable sources. One of the main potential applications for 2,5-furandicarboxylic acid is the replacement of plastic bottles polyethylene terephthalate, commonly known as PET. More specifically, 2,5-furandicarboxylic acid units can be polymerised to yield polyethylene 2,5-furandicarboxylic acid (PEF), a bio-based polymer with similar properties to fossil fuel-based equivalent. Further applications of HMF include the production of levulinic acid, another biofuel precursor, and 2,5bis(hydroxymethyl)furan, a feedstock in the manufacturing process of polymers such as polyesters and polyurethane foams.

1.5 Integrated biorefineries

1.5.1 Concept within the literature

According to the International Energy Agency (IEA) a biorefinery is “the sustainable processing of biomass into a spectrum of marketable products and energy” (118). An integrated biorefinery can take more than one type of feedstock to be processed through different possible configurations of processes yielding several products. Each biorefineries will have its unique configuration depending on both the feedstock and main desired products. One of the main characteristics of integrated biorefineries is the creation of as little waste as possible. Therefore, the residues obtained from the many biorefinery conversion units will be further processed and converted into other products and/or intermediates. Ahring et al were amongst the first researchers to use the concept of “integrated biorefinery” in 2005 when the authors designed a plant to produce biofuels and chemicals from lignocellulosic biomass. Since then numerous studies have been published. The feedstock used in biorefineries can be from different sectors such as forestry, industry, agriculture, municipal waste and aquaculture.

An integrated biorefinery is a facility that uses mechanical, chemical, thermochemical and biochemical processes to convert the biomass into high value products with maximum energy and material recovery (119). In other words, it is a biorefinery that utilises the biomass and power to its maximum extent with little or minimal losses. It does so by trying to reuse the “wastes” of the main processes in secondary processes and convert it into by-products and if possible to use the wastes of these secondary processes in other reactions until no more added value products can be obtained from the waste streams. In addition to the maximum material recovery, a maximum energy recovery can also be employed. This requires an assessment of the biorefinery to determine in which operations the energy can be harvested to be reused in other processes. This allows the biorefinery to reuse energy that would otherwise be wasted, instead of using new energy from the grid that would incur in higher costs. Such systems are also known by integrated energy recovery systems. These changes turn the biorefinery into a more sustainable, self-dependent and lower emission facility equipped with the tools to convert biomass into a variety of final products at minimal cost and high efficiency. The inclusion of these integrated recovery systems also leads to a positive economic impact on the biorefinery.

The increased interest in integrated biorefineries is shown by the growing number of publications including the words “integrated biorefinery” in the last decade (Figure 1-2). This is particularly relevant from 2012 until 2017 which corresponds to the period where

there was a significant increase in the number of publications. Previously to 2012 the number of studies on integrated biorefineries are relatively constant when compared to the year before. Even though the trend has been decreasing slightly since 2017, 26 research studies were published in 2019 (higher than any year previous to 2017, except for 2014). This demonstrates that integrated biorefineries are still a hot topic in the research community, with 2020 set to be the most productive year yet (Figure 1-2) for articles using integrated biorefineries for biofuel production.

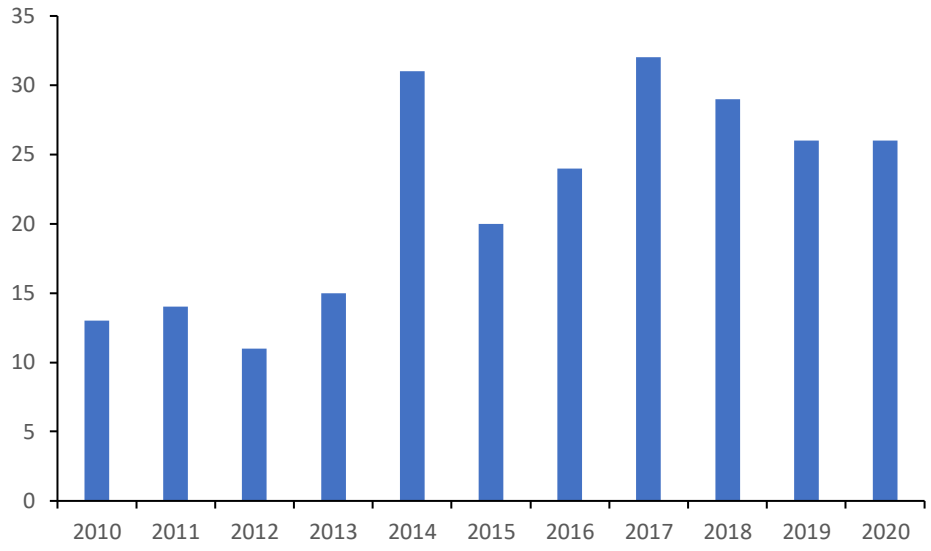

Figure 1-2 – Number of publications per year including the words “integrated biorefinery” in the last decade

The studies found in the literature were also grouped into four different types: review article, techno-economic, experimental and modelling & simulation. Figure 1-3 shows the percentages of these groups in the total number of studies found. Experimental studies have a much higher significance when compared to the other group of studies. This suggests that research has been focusing on novel and alternative pathways to produce biofuels on a sustainable manner rather than reviewing, studying the techno-economics and modelling the existing pathways.

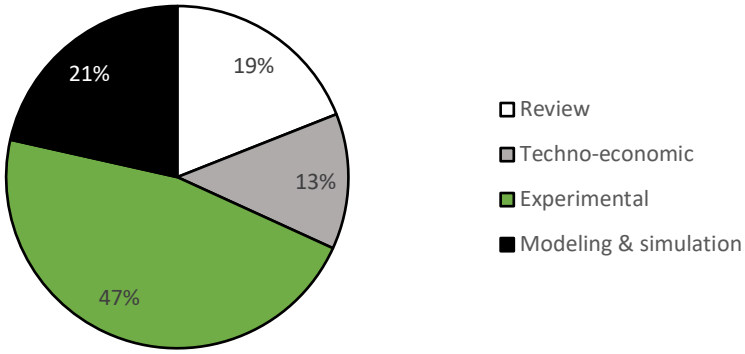

Figure 1-3 – Percentages of type of studies found in the literature including the words “integrated biorefinery”

1.5.2 Integrated biorefinery processes grouped around the initial feedstock used

A huge range of feedstocks are available for processing in a biorefinery concept. These range from traditional sources such as wood and agricultural crops residues to less developed sources such as microalgae or municipal wastes that would otherwise have to be disposed of (Figure 1-4). Agricultural residue waste corresponds to a large portion of the total feedstocks used, with over a third of the experimental studies in integrated biorefineries using this type of feedstock. The remaining feedstock types has a relatively similar percentages falling between 10% and 15% with forestry biomass the top one and algae the least used one.

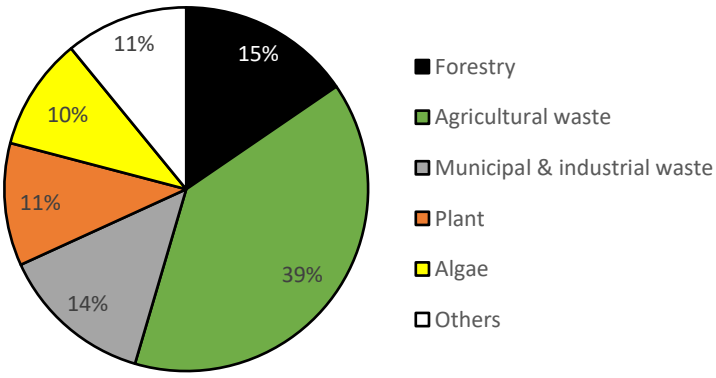

Figure 1-4 – Significance of each type of feedstock in the experimental publications that include “integrated biorefinery” in their title

1.5.2.1 Integrated biorefineries using forestry feedstock

The main industry valorising wood is arguably the paper and pulp sector. In the last decade, a number of authors have investigated the integration of paper production with biofuel development. For example Zhang et al investigated an aeration process capable to be included in pulp mill to extract manool from black liquor (120). Ajao et al demonstrated the production of biobutanol by using an alkaline pretreatment to cook yellow birch chips at 170 °C in a mixture of sodium hydroxide, sodium sulphide and water to yield a cellulose pulp (121). This pulp was then followed by an enzymatic hydrolysis with cellulase and xylanase to depolymerise the oligosaccharides into fermentable sugars. These sugars were finally fermented using a *Clostridium acetobutylicum* strain to yield biobutanol under anaerobic conditions. Guigou et al have also used a kraft pulping in wood from *Eucalyptus grandis* (122). However, the biomass was initially processed in a hemicellulose extraction with green liquor at a temperature of 155-160 °C during two and a half hours. The extracted hemicelluloses were further processed in a fermentation with *Scheffersomyces stipitis* to produce bioethanol with a sugar conversion yield of 89%. The resulting hemicellulose-free biomass was the fed into a kraft pulping process in a pulp mill industry. Mendes et al also performed a hemicellulose extraction but from another strain of eucalyptus (*Eucalyptus globulus*) chips using two pretreatment steps (123). Such extraction allows the hemicellulose to be further processed and converted into value-added products, instead of being submitted to the cooking stage in a paper pulp mill and dissolved in the black liquor that is burnt for energy recovery. The two pretreatment steps consisted of an auto-hydrolysis and acid-hydrolysis. The auto-hydrolysis consists on the heating of the wood chips dissolved in water up to 150 °C for a period of 2 to 3 hours. The acid-hydrolysis was performed after the auto-hydrolysis and uses sulphuric acid to hydrolyse the oligosaccharides into monosaccharides.

Li et al investigated the impact of shortening the duration of the kraft pulping duration on woods with high kappa number (124). This can be done as the woods with a high kappa number have a higher concentration in lignin, which provides an increased recalcitrant nature to cellulose. However, the oxygen delignification process performed downstream to the kraft pulping can remove the lignin from the pulp and improve energy savings, generation of methanol and better biomass utilisation. The lignin removed from this pulp can then be used in the production of value-added products.

It was found that many of the studies using forestry feedstocks in a biorefinery context focus on the lignin extraction as one of the core processes in the biorefinery. Chen et al studied a catalytic fractionation process to pretreat wood from *Eucalyptus grandis* (125). In this pretreatment step a Pd/C catalyst was used to separate the biomass components

into two main streams: lignin and carbohydrates. This pretreatment step also works as a depolymerisation process with 4-propanol guaiacol and 4-propanol syringol being the main phenolic products found in the lignin stream. The carbohydrates stream was submitted to further catalytic processing with a $FeCl_3$ catalysts to produce 5-HMF, furfural and levulinic acid.

Yoon and Heiningen used fresh green liquor to extract carbohydrates and lignin after the pretreatment of green pine (126). This extraction was performed at an alkaline pH to neutralise the acidic content induced by the acids released upon pretreatment. The green liquor is a sodium oxide solution with percentages ranging from 2 to 6% being studied. More concentrated green liquors led to increased yields of wood extracts obtained. The authors quantified the carbohydrates and lignin extracted with the yield depending on the temperature and pretreatment residence time. The extracted carbohydrates and lignin can be further upgraded into value-added products.

Toledano et al used an organosolv fractionation process with a mixture of ethanol and water to extract lignin from olive tree wood (127). This lignin showed a low contamination of hemicellulose and depolymerised hemicellulose depending on the severity of the organosolv pretreatment. The fractionation process also provided a stream rich in cellulose that can be further processed into alternative products. Romani et al also developed a organosolv treatment to delignify *Eucalyptus globulus*, however instead of the solvent mixture of ethanol and water, the authors used a mixture of glycerol and water (128). The organosolv pretreatment was performed at 200 °C for 69 minutes. The authors found the same problem as Toledano et al, where the lignin stream was found to be contaminated with hemicelluloses. However, in this case, the lignin was separated by precipitation with hydrochloric acid followed by centrifugation. The delignified wood stream, mainly rich in cellulose, was washed with sodium hydroxide to neutralise the acidic pH of the solution due to the acids released during the pretreatment step. This was followed by a water wash, a saccharification into fermentable sugars and fermentation with *Saccharomyces cerevisiae* to produce bioethanol.

Hundt et al studied the alkaline polyol pulping (AlkaPolP) which fractionated the biomass into different streams (129). The authors proved the applicability of the process in various types of feedstock such as hardwood, softwood and grass-type. The process delignifies the biomass, which is then depolymerised by cellulases for further processing in the fermentation processes.

Tian et al used the recalcitrant lignin (lignin residue obtained after the hydrolysis of feedstock) from corn stover, softwood and hardwood to produce lignin nanoparticles

(130). This product has gained increased interest in the last years due to its application in the biomedical and environmental fields. This study fits well into a complete biorefinery design as lignin is commonly discarded without further valorisation (131–133).

Other integrated biorefinery approaches using woody biomass presented by Cebreiros et al used different strains of *Eucalyptus* and considered the pretreatment of this feedstock as well as the downstream processes and valorisation of every stream. An integrated biorefinery using Eucalyptus sawdust was therefore developed (134). This study comprised two possible approaches. The first approach includes an organosolv pretreatment with ethanol at 180 °C for 45 minutes to yield a solid and liquid fraction. The solid fraction was then submitted to enzymatic hydrolysis to yield a stream rich in glucose. The liquid fraction was fed into an ethanol organosolv precipitation to extract lignin. The resulting stillage from this process was mainly xylo-oligosaccharides, xylose and acetic acid. On the second approach the Eucalyptus sawdust was hydrolysed in an autohydrolysis pretreatment also at 180 °C for 45 minutes. The liquid fraction was rich in xylose, oligosaccharides and acetic acid, with some furfural and glucose content. Just like in the first approach, the solid fraction was submitted to an enzymatic hydrolysis yielding a glucose rich stream. In both enzymatic hydrolysis a non-ionic surfactant was added to improve the hydrolysis results. The products of this biorefinery can be further converted into high-value products.

1.5.2.2 Agricultural residues for integrated biorefineries

The majority of research into integrated biorefineries use agricultural residues as a feedstock, with over 20 different feedstocks developed.

Sugarcane

One of the most used types is the waste obtained from the sugar cane agriculture. Sugarcane was among the first feedstocks used in the production of biofuels. There is a clear driver to increase value from both the first generation bioethanol production and crystalline sugar production. As such the valorisation of sugarcane bagasse, and other wastes obtained after the processing of sugarcane has been heavily discussed. For example, in 2015 Ghosh et al studied an integrated biorefinery that produces bioethanol, furfural and electricity from sugar cane bagasse (135). Acid hydrolysis and enzymatic saccharification were used to yield glucose and xylose. These hexoses were then used in the fermentation for bioethanol production using *Kluyveromyces sp.* while the pentoses were used in the production of furfural through a biphasic catalytic dehydration process (aqueous stream containing the pentoses and MIBK phase). The enzyme used in the enzymatic saccharification was recycled for further use in the same process. The

residual stream, rich in lignin, was pelletised and then fed into a gasification process for electricity production.

Sugar cane bagasse has also been used in the production of levulinic acid (136). The bagasse was pelletised previous to the pretreatment with liquid hot water at 200 °C for 30 minutes. This was followed by an enzymatic hydrolysis yielding a sugar stream rich in glucose (44 g/L) and xylose (11.7 g/L) and with vestigial concentrations of acetic acid and furfural (2.6 and 0.4 g/L, respectively). This stream was finally submitted to a dehydration process using methane sulfonic acid as an acid catalyst to produce levulinic acid. The authors studied different temperatures, residence times and catalyst concentrations for higher levulinic acid yields. It was determined that the highest levulinic acid yield (67%) was obtained at a temperature of 206 °C, a reaction time of 30 minutes and an acid concentration of 0.63 M.

Shifting from sugar cane bagasse to sugarcane stalks, later in 2019 Sartori et al demonstrated a fractionation process to separate this feedstock into two streams: an outside part which is denser and the core of the stalks (137). The outside part of the stalks was used in the production of steam and power, while the core of the stalks was further treated for sucrose extraction. This stream showed a 34% higher utilization of polysaccharides when compared to the sugar cane bagasse.

The studies performed from Santos et al and Martinez-Guido et al have proposed integrated biorefinery approaches in close proximities to sugarcane industries to use the waste streams produced in this industry (138,139). In 2014 Santos et al proposed a supercritical fluid extraction facility (138). This facility was designed to use the ethanol, electricity and carbon dioxide produced in the sugarcane biorefinery to yield bioactive compounds such as β-ecdysone. The integration of the supercritical fluid extraction facility near to the biorefinery resulted in the reduction of the utilities required in this new plant. One year later Martinez-Guido et al presented a comprehensive study to integrate a biorefinery in a sugar cane industry (139). The authors suggested retrofitting a sugar cane facility by reusing some of the waste streams from the sugar cane industry in a biorefinery to produce bioethanol. The biomass was initially pretreated to yield a sugar juice which then went through a combination of filtration, purification and crystallisation processes to yield sugar. During the sugar production process, some waste streams can be recovered and fully upgraded into value added products. One of these streams was used in the electrical plant to produce steam which was recycled to the sugar cane plant and used in the evaporation processes. Another stream called “cachaza” can be used both in the production of biofertilizer and cattle feed. Finally, a stream of bagasse

collected from the production of juice sugar was directed to the biorefinery for the production of bioethanol.

A similar feedstock to sugarcane is sugar beet which is also used in the production of sugar due to its high content of sucrose. The processing industry of sugar beet leads to enormous quantities of sugar beet pulp. This by-product has the potential to be used in a biorefinery concept due to its high carbohydrate-content. In 2017 Cardenas-Fernandez et al assessed the applicability of sugar beet pulp in an integrated biorefinery (140). The design of this biorefinery consisted in the fractionation of the feedstock using a steam explosion treatment to separate pectin from a solid cellulose-rich stream. This solid stream was submitted to an enzymatic hydrolysis to produce monosaccharides which were used in a downstream fermentation to yield bioethanol. The pectin fraction can be partially or fully hydrolysed for subsequent enzymatic upgrading to L-gluco-heptulose. This product has therapeutic applications in the medicine field in cancer and hypoglycaemia. In 2018, Cardenas-Fernandez et al presented a second publication that studied the hydrolysis of sugar beet pectin using a α-L-arabinofuranosidae to produce L-arabinose and galacturonic acid rich backbone. The hydrolysis was performed using immobilised α-L-arabinofuranosidae in a packed bed reactor, which can be run for 7 days with a minimum 73% performance. The L-arabinose and galacturonic acid were separated by tangential flow ultrafiltration yielding an L-arabinose-rich stream with 92% recovery of this monomer.

Similarly, Suhaili et al used other wastes from the processing of sugar beet: sugar beet vinasse (141). The authors demonstrated the production of an enzyme (CV2025 ω-Transaminase) from sugar beet vinasse. The main application of the biocatalyst produced is on the production of amines from aldehydes and ketones (142).

Residues from corn processing

Another common feedstock used in integrated biorefineries are the waste biomass associated with corn food production. The waste streams include corncob, corn straw, corn stalks and corn stover. Han et al studied a biorefinery system that used corncob as feedstock to obtain glucose and a stream of xylooligosaccharides (143). The process started with a pretreatment using gluconic acid that is also a hydrolysis of the carbohydrates in corncob. The authors used a response surface methodology to optimise the xylooligosaccharides yield obtained from the pretreatment. This pretreatment yielded a solid fraction rich in glucose and a liquid fraction rich in xylooligosaccharides with an 18% yield. The solid fraction was further hydrolysed using cellulases from where 290 g of glucose was obtained from 1 kg of corncob.

Corncob was also used as a feedstock to produce a biodiesel, bioethanol and lignin-based sunscreen (144). The feedstock was pretreated using a solution of 1% of sulphuric acid to yield a liquid stream rich in hemicellulose hydrolysates and a solid stream. The liquid stream was fed into a yeast (*Trichosporon cutaneum*) fermentation from where microbial lipids were obtained. Such lipids were submitted to a transesterification using an alkali catalyst to produce biodiesel. The resulting solid residue after pretreatment was submitted to an enzymatic hydrolysis from where a liquid stream rich in glucose was obtained. This stream was used in a fermentation process with *Saccharomyces cerevisiae* to yield bioethanol. Finally, the lignin-based sunscreen was obtained by adding the resulting solid stream after the enzymatic hydrolysis to a carrier lotion.

In 2018 Gandarias et al studied an innovative integrated biorefinery approach that used corncob to produce 2-methylfuran (145). The corncob was initially mechanically processed and fed into a pretreatment step at 200 °C without any catalyst, process usually called an auto-hydrolysis pretreatment to yield a sugar liquor. The authors mentioned that such pretreatment conditions are designed for a higher production of C5 sugars rather than C6, which allowed the extraction of these sugars in two separate streams. This separation allowed higher overall yields of furfural and HMF which were produced from C5 and C6 sugars, respectively. The production of furfural was performed through a catalytic dehydration of the C5 fraction using a solid acid catalyst. The produced furfural was subsequentially separated from the resulting aqueous solution using an organic mixture of 5-methyltetrahydrofolate, toluene and 2,5-dimethylfuran.

In 2020 Li et al presented a biphasic pretreatment process with methyl isobutyl ketone and water to fractionate and convert corn stalk into furfural and residual streams (146). The results show that furfural reached a 52% yield, with only 2.3% of hemicelluloses left in the residue. The residues showed a high content in lignin and glucan and when submitted to an enzymatic hydrolysis resulted in a glucose yield of 85.5%. This stream can be used in a fermentation process to yield bioethanol. The residue stream from the enzymatic hydrolysis was rich in lignin and is a by-product of this biorefinery design.

Corn stover was used in an integrated biorefinery designed by Yang et al where the authors demonstrated that an acidic steam explosion pretreatment followed by an alkaline ethanol post-treatment of this feedstock leads to a cellulose rich-enriched stream (147). An increase in the concentration of sodium hydroxide from 0.05% to 4% in the alkaline ethanol post-treatment resulted in reduced lignin content in this cellulose-enriched fraction of 10.7% (reduction from 32.8%). The cellulose-enriched fraction was then submitted to an enzymatic hydrolysis to yield monosaccharides which could be used

in the production of bioethanol through fermentation (not performed in this study). The separated lignin fraction after the alkaline ethanol post-treatment and the solid fraction after enzymatic hydrolysis were further upgraded into lignin-phenol-formaldehyde resins.

Later in 2020 Liu et al have gone further and developed an integrated biorefinery entailing an enzyme-free mechanocatalytic saccharification on corn stover and a subsequent fermentation of the produced fermentable sugars into bioethanol (148). The enzyme-free mechanocatalytic saccharification consisted of a combined milling of the feedstock, corn stover, with phosphorus pentoxide, acting as a catalyst, followed by hydrolysis into fermentable sugars. The authors claim that this design overcomes the common problems associated with liquid phase and solid catalysts to convert lignocellulosic biomass to glucose. Lignin was also recovered as a by-product in this integrated biorefinery approach.

Corn straw was the feedstock used in an integrated biorefinery presented by Xia et al which used an acid pre-impregnated steam explosion to pretreat the biomass (149). In this process, the biomass was immersed in a diluted solution of sulphuric acid for a period of time (30 minutes in this case) followed by filtration for acid recovery and its reutilisation in the acid immersion. The solid fraction was subsequently submitted to a steam explosion from where the effluent was washed with water. A pulp and an aqueous fraction were obtained. The pulp fraction was used in the production of a hand sheet, while the aqueous fraction was concentrated and fermented with *Clostridium acetobutylicum* to obtain butanol.

Wheat straw

In addition to the straw from corn, straw obtained from wheat was also studied by other researchers. Budarin et al presented an integrated biorefinery that consisted of a supercritical carbon dioxide extraction to remove lipids from wheat straw and a low temperature pyrolysis by microwave treatment (150). The authors claim that a supercritical carbon dioxide extraction did not leave any solvent traces in the products which makes them suitable for food, pharmaceutical and cosmetics industries. The streams obtained after pyrolysis such as gas fraction, bio-oil and biochar were further used in the biorefinery design. The gas fraction was used for power generation. The bio-oil was submitted to a water treatment which separated it into an organic fraction, used in the production of chemicals and power generation, and in a sugar stream to be fed into biochemical processing, such as fermentation for bioethanol production. Finally, the solid char was submitted to an aqueous extraction of the organic compounds that can be fed into the biochemical processing, while the inorganic compounds were further used

in power generation. This biorefinery design used several integrational pathways, recovering energy and material (such as carbon dioxide) to be reused within the biorefinery units.

Morais et al suggested the optimised production of a glucan stream from wheat straw in an autohydrolysis at high CO_2 pressure (up to 60 bar) (151). The process yielded oligosaccharides and increased the digestibility of cellulose to be subsequently processed in an enzymatic hydrolysis. Both the autohydrolysis and the enzymatic hydrolysis with cellulase resulted in a glucose yield of 82% from glucan.

Nuruddin et al and Jahan et al characterised different straw feedstocks and assessed their use and impact in the proposed biorefinery designs. Nuruddin et al assessed the composition of rice straw, wheat straw, corn stalks and dhaincha (152). The feedstocks were fractionated with formic acid, peroxyformic acid and peroxide followed by an acid hydrolysis. The authors demonstrated that dhaincha and corn stalks have higher content of cellulose (42.8 and 43%, respectively), while rice straw and corn stalks have higher percentage of hemicellulose (32.9 and 31.2, respectively) and dhaincha has higher content in lignin (19.7%).

Jahan et al assessed several agricultural waste feedstocks in an integrated biorefinery design towards the production of dissolving pulp (153). The authors performed a characterisation of the agricultural wastes studied (rice and wheat straw, lentil stalks, mustard branch and stem) previous to its treatment. These feedstocks showed relatively similar compositions: cellulose ranging from 35 to 40% and a lignin content in the range of 23 to 26%. However, the ash content goes from 4% and 5% in lentil stalks and mustard branch up to 15% in rice straw. The feedstock was initially pretreated in a prehydrolysis step from where 32 to 39% of the biomass was dissolved into a liquor. This liquor was mainly composed by lignin, acetic acid, oligo and monosaccharides. It was further used in the production of bioethanol by fermentation or alternatively in the production of other high value products such as furfural. The presence of acetic acid in the prehydrolysis liquor acted as an inhibitor in the fermentation process. Therefore, its separation from this stream led to an increase in the bioethanol yield. This separation can be performed by adsorption or liquid-liquid extraction, being the acetic acid one of the by-products of this biorefinery design. The resulting biomass after the prehydrolysis was then cooked through a kraft process to produce pulp. The pulp yields are relatively low, ranging from 30 to 38%, being the lowest yield corresponding to wheat straw and the highest to lentil stalks. The pulps were subsequently bleached achieving relatively high brightness (87% to 90%).

Residues from the edible oil industry

Another agricultural waste frequently used in integrated biorefinery studies is the waste streams obtained after the processing of palm oil biomass. In 2015 Ali et al proposed a biorefinery to reuse the waste after a palm oil mill industry to produce a wide variety of high value products (154). Wastes such as empty fruit bunch, mesocarp fibre, palm kernel shell and palm oil mill effluent are proposed as feedstock to be used in the biorefinery. Later in 2017 Yusof et al demonstrated a bioethanol based biorefinery design using oil palm frond (155). The feedstock was pretreated using a wet disc mill, from where 80% of holocellulose was recovered. 95% of the recovered holocellulose was converted to glucose and xylose in a saccharification. The produced sugars were finally fermented using a culture of *Saccharomyces cerevisiae* to yield bioethanol. The produced bioethanol was finally distilled and purified to obtain a product with 98.9% purity. In 2019 Burhani and Triwahyuni have also assessed an integrated biorefinery to produce bioethanol but this time using empty fruit bunch from palm oil (156). In this study the holocellulose is saccharified and fermented with *Saccharomyces cerevisiae* to produce bioethanol while the lignin was extracted with black liquor, followed by purification and upgrading to flame retardant additives.

The production of olive oil generates four times the amount of waste as it generates of olive oil which poses a disposability problem of this waste. However, this can be used in the production of biofuels as it was demonstrated by Schievano et al when they used pomace in a supercritical carbon dioxide extraction using ethanol as a polar co-solvent to extract polyphenols and fatty acids (157). The remaining biomass fraction can be either fed into a combustion for heat and power production, or to a pyrolysis for bio-oil and biochar production. Later in 2018 Goldfarb et al presented an integrated biorefinery that intends to tackle the disposal problem of olive waste (158). In addition to the supercritical carbon dioxide extraction of polyphenols and lipids presented by Schievano et al, Goldfarb et al suggested that the resulting solids can be further processed in a pyrolysis unit to yield bio-oil and biochar. The study also showed that the biochar can be used in an activation process for application in water treatment systems, removing heavy metals from water. This study demonstrates that both carbon dioxide activation and potassium hydroxide activation processes are suitable for the production of activated carbon.

Other authors opted for less common feedstock sources such as cottonseed (Zhu et al (159)), vine shoots (Davila et al (160)), sunflower (Kachrimanidou et al (161)), soybean (Sekhon et al (162)) and horse chestnut burs (Gullon et al (163)). The integrated biorefinery approach presented by Zhu et al shows a two-phase extraction on cottonseed

(159). This extraction was performed at 40 °C for a period of 20 minutes and with the help of petroleum ether and methanol in a ratio of 1:3. The process generated three streams from cottonseed: an upper phase, a lower phase and nontoxic cottonseed meal as denominated by the authors. Crude sterol, gossypol and raffinose were the products obtained from the lower phase through a combination of separation processes such as crystallization and numerous evaporations. The upper phase was submitted to a supercritical methanol reaction from where the products were separated by crystallization yielding a crude sterol stream and a residual solvent stream. The crude sterol was recrystallised which resulted in the sterol final product, while the residual solvent went through a separation from where a glycerine phase and a biodiesel phase were obtained. Glycerine and biodiesel were obtained by refining of these two phases.

In 2015 Kachrimanidou et al studied the fractionation of sunflower meal (residue after the sunflower oil extraction) in water to yield an aqueous fraction, a protein-rich fraction and a lignocellulosic-rich fraction (161). The protein-rich fraction was submitted to an alkaline extraction followed by an acid precipitation to yield a protein isolate with 94% purity. The lignocellulosic-rich fraction was submitted to a solid state fermentation with *Aspergillus oryzae*. This was then combined with the aqueous fraction and the residue of the protein fraction to be used in an enzymatic hydrolysis. The product of this hydrolysis is further processed in a microbial bioconversion to produce poly(3-hydroxyburtyrate). The bioreactor was set at 30 °C, with a pH between 6.7 and 6.9 and using an air flow rate of 1 vvm (airflow unit in a bioreactor). The authors proposed the integration of this design in a sunflower-based biodiesel biorefinery leading to the recover and utilisation of the residues from the production of sunflower oil. In 2016, Kachrimanidou et al demonstrated that poly(hydroxyalkanoates) can be recovered from cells of a bacteria (*Cupriavidus necator*) (164). These cells were lysed by crude enzyme produced in the solid state fermentation in their integrated biorefinery design in 2015 (161).

In 2016 Davila et al presented an autohydrolysis process on vine shoots at 201 °C that yielded a solid phase and a liquid phase rich in oligosaccharides, monosaccharides and other minor compounds (160). The solid stream, rich in cellulose and lignin, was further studied in a second publication in 2017, in which a delignification process was also carried out (165). This delignification was performed using a solution of 12% of sodium hydroxide at 124 °C for a period of 105 minutes. These conditions led to an extraction of 67% of the lignin present in the solid phase into a liquid phase from where the lignin was recovered after precipitation. The resulting delignified solid stream had a glucan content of 69.4%. This stream was subsequently submitted to an enzymatic hydrolysis from

where a glucose-rich liquid phase was collected, while the remaining solid phase was mainly composed by lignin. The combination of these two studies by Davila et al forms what can be seen as the initial treatment in an integrated biorefinery to produce intermediates. These intermediates, glucose and lignin, can then be used in upgrading processes to produce biofuels and high value chemicals.

Sekhon et al presented a study where insoluble fibre from soybeans were used as a feedstock in an integrated biorefinery to produce bioethanol (162). The insoluble fibre was obtained from soybeans by an enzyme-assisted aqueous extraction process. This stream was rich in proteins and carbohydrates. The authors assessed five different designs to produce bioethanol. The first design used a soaking aqueous ammonia pretreatment at 80 °C for 12 hours as a pretreatment followed by saccharification and fermentation. The second design replaced the soaking aqueous ammonia pretreatment by a liquid hot water pretreatment (at 160 °C for 20 minutes) and maintained the saccharification and fermentation. In both of these designs the saccharification was carried out at 50 °C for 2 days and the fermentation occurred at 35 °C over 48 hours using *Saccharomyces cerevisiae*. The third design consisted of a single process comprising a simultaneous saccharification and fermentation (*S. cerevisiae*) at 37 °C for a period of 3 days. The fourth design used the same simultaneous saccharification and fermentation with *S. cerevisiae* but added a second simultaneous saccharification and fermentation. However, the bacteria used in this second fermentation was *Escherichia coli*. Finally, the fifth design added a liquefaction process to the fourth design previous to the first simultaneous saccharification and fermentation. The authors concluded that the saccharification of the insoluble fraction with enzymes (a mixture of pectinase, cellulase and xylanase) led to better bioethanol results when compared to the soaking aqueous ammonia and liquid hot water pretreatments. The authors have also demonstrated that the combination of the insoluble fibre with corn increases the ethanol production rate while oil recovery of the stillage after fermentation.

Dried distillers grains and solubles (DDGS)

Dried distillers grains are other waste that was found in more than one integrated biorefinery publication. In 2014 Lupitskyy et al presented a study using dried distillers grains in a hydrolysis that served as a pretreatment step to produce a xylose-rich stream (166). In addition to this stream, two co-product streams were also generated from the mechanical treatment of the feedstock (fine fraction) and the pretreatment (residual fibre). The authors determined that the first stream had a higher content in protein while the second stream was richer in lipids. Both these streams could be used in animal feed as additives. In the same year Fonseca et al demonstrated that the high hemicellulose

content in dried distillers grains can be an advantage in an integrated biorefinery concept (167). An acid hydrolysis of the dried distillers grains was performed in a percolation reactor with liquid recirculation. A two-stage hydrolysis process was carried out which allowed an arabinose and a xylose-rich streams (81.5% and 85.2%, respectively). This design led to a pentose yield of 94%. This study could be employed as an initial pretreatment step in an integrated biorefinery using the dried distillers grains where the two pentose streams can be further upgraded to biofuels and high-value chemicals.

1.5.2.3 Municipal and industrial food wastes

One huge source of biomass, in addition to the agricultural residues, is food waste disposed of in urban areas, by supermarkets due to spoilage or as a by-product of industrial food processing.

One large food waste type are the peels obtained from fruits and vegetables. In 2014 Naranjo et al proposed various biorefinery designs using banana waste, both pulp and peel, in the production of polyhydroxybutyrate as the main product (168). The first scenario was a straightforward design only processing the banana pulp in a hydrolysis and fermentation conversion processes to yield polyhydroxybutyrate. The second scenario added an enzymatic hydrolysis step to the peel waste, producing mainly glucose and xylose. While xylose was exclusively used in the production of polyhydroxybutyrate, glucose was partially used in the production of polyhydroxybutyrate but also in the production of bioethanol. The pulp hydrolysis consisted of a liquefaction (carried out at 90 °C during 3 hours using alpha amylase) and a saccharification (run at 70 °C for 2 days) using glucoamylase. The banana peel hydrolysis process used an autohydrolysis process (liquid hot water at 170 °C) followed by saccharification of cellulose by cellulases. The production of polyhydroxybutyrate and ethanol was performed by fermentation processes using *Burkholderia sacchari* and *Pichia stipites*, respectively, as the microbial agents. The third scenario used the second scenario but also included a mass and energy integration. The energetic integration led to a reduction of 31% in the energy used while the mass integration saved up to 35% of water.

In 2018, Talekar et al applied a hydrothermal treatment to pomegranate peels waste (169). In this study the authors studied the hydrothermal treatment using a range of temperatures from 100 to 130 °C, different liquid-solid ratios and a treatment duration ranging from 20 to 60 minutes. This treatment resulted in a recovery of three streams: a pectin-rich stream, phenolics-rich stream and a residue from this process. The optimal hydrothermal treatment conditions were found at 111 °C, 12.5 liquid-solid ratio and 39.5 minutes, resulting in a 20.9% and a 11.9% pectin and total phenolics yields, respectively.

The residue from this hydrothermal treatment was subsequently submitted to an enzymatic hydrolysis into fermentable sugars which were then fermented to yield 80 g of bioethanol per kg of biomass. This resulted in an 88% bioethanol yield from the 177 g of glucose produced per kg of biomass processed.

In 2019 Dlangamandla et al demonstrated the hydrolysis of peels from *Citrus sinensis* and *Malus domestica,* cobs from *Zea mays* and *Quercus robur* using *Nepenthes mirabilis* (commonly known as tropical pitcher plant) digestive fluids (170). The authors determined that these digestive fluids present a good hydrolysis potential of holocellulose and phenolic compounds due to their content in carboxylesterases, β-glucosidases and xylanases. Such process presents the potential to be used within an integrated biorefinery approach.

In 2020 Tsouko et al developed an integrated biorefinery to extract free sugars, essential oils, a phenolic extract and pectin also from orange peels (171). The remaining stream was further processed in a chemical and enzymatic hydrolysis to yield a hydrolysate rich in sugars. This stream was then used in the production of bacterial cellulose by fermentation using *Komagataeibacter sucrofermentants*. The free sugar stream was also submitted to this fermentation using the same bacteria. A process design was also performed from where it was determined that 62% of energy requirements could be saved.

Similarly, Ebikade et al demonstrated an integrated biorefinery approach to repurpose potato peel waste (172). In this approach the potato peel waste was initially submitted to an ultrasonic extraction using methanol and water in a ratio 50:50 to extract antioxidants such as chlorogenic acid and caffeic acid. The compounds that were not extracted were then processed in a hydrolysis and simultaneous dehydration of the sugars into 5-hydroxymethylfurfural with a yield of 54%. The effluent from this reaction was treated in a liquid-solid separation. The HMF was extracted from the liquid phase in a separation with an organic solvent. The HMF was collected from the organic solvent by distillation while the organic solvent was recycled by distillation. Finally, the solid phase recovered from the liquid-solid separation, mainly rich in residual lignin, was submitted to a pyrolysis reaction yielding biochar.

Zhang et al have used another municipal waste such as green tea leaves to demonstrate an efficient extraction and recovery of protein from this feedstock (173). The process consisted of a pretreatment using ethanol as a co-solvent, a mild alkaline treatment, protein extraction, hydrolysis and fermentation. The ethanol pretreatment (ethanol recycling) resulted in the extraction of phenols, lipids and organic acids. The alkali

treatment resulted in a stream rich in pectins and some protein. However, most of the protein was recovered on the protein extraction process (75%) with some lignin, pectin and residual amounts of hemicellulose. The residue stream resulting from the protein extraction was rich in carbohydrates and had the potential to be submitted to a hydrolysis followed by fermentation. The authors suggested that pectin is possibly one of the most crucial constraint towards the efficient extraction of protein from leaves.

In addition to the waste obtained from municipalities, researchers have also accessed the use of waste streams obtained from industrial activities. In 2014 Ichsan et al used a waste stream obtained from a palm mill oil facility, while other used waste streams from pulp paper mills, and Rosberg et al repurposed the carbon dioxide obtained from a bioethanol biorefinery.

Ichsan et al studied the suitability of an industrial waste water such as palm mill oil effluent as a growth media for microalgae (174). This effluent was demonstrated to be a suitable feedstock in the production of biogas by anaerobic digestion. However, the authors have proved that it can also be used in the growth of microalgae (*Spirulina sp., Chlorella sp.* and a wild algae strain). This study fits in an integrated biorefinery approach as the produced algae can then be used in the biorefinery for the production of biofuels and energy. The study presented by Chavan and Mutnuri goes in line with the work presented by the previous authors (175). In this study *Spirulina platensis* was grown using the nutrients present in domestic wastewater.

The work presented by Alexandri et al studied two different integrated biorefinery designs using the spent sulphite liquor from a pulp paper mill (176). Both these designs include the extraction of lignosulphonates with organic solvents (isopropanol and ethyl acetate in the first and second design, respectively). The second design also included a nanofiltration step previous to the lignosulphonates extraction to yield a fraction rich in carbohydrates and free from toxic compounds. The resulting stream after extraction was further used in fermentation processes using *Actinobacillus succinogenes* (in the first design) and *Basfia succiniciproducens* (in the first and second design) to produce succinic acid. Both the designs demonstrated this waste from pulp paper mills can be integrated in a biorefinery towards the production of value-added products.

Zambare and Christopher assessed the hydrolysis of primary sludge into fermentable sugars (177). Primary sludge is the solid waste recovered after the effluent treatment on a pulp and paper mill industry. The authors studied the hydrolysis variables such as reaction time, enzyme, solids and surfactant amount to improve hydrolysis yield. Another studied variable in this biorefinery includes enzyme recovery. The optimal conditions

yielded a hydrolysate that was then fermented using *Saccharomyces cerevisiae* to produce bioethanol with a yield of 92% and *Cutaneotrichosporon oleaginosum* resulting in a biolipid yield of 38%. The authors have also achieved a 40% enzyme recovery by centrifugation and ultrafiltration of the hydrolysate. The reuse of the primary sludge not only allows the production of value-added product but also contributes to the reduction of disposal costs.

Rosenberg et al studied the use of carbon dioxide and waste heat obtained from a bioethanol biorefinery in the growth of microalgae (*Chlorella vulgaris*) (178). The grown microalgae can then be used in the biorefinery towards the production of bioethanol.

Spent coffee grounds

Coffee is the second most traded commodity only after oil (179). The yearly consumption of coffee beans in 2020 was estimated at 9.8 million tonnes and the estimations predict a consumption of 10 million tonnes in 2021 (180). The coffee beans are roasted and ground to the correct size (depending on the brewing process). The coffee beans brewing process can happen at different temperatures and pressures, depending on the brewing process (pressure, infusion or filtration). The brewing process extracts part of the components present in the coffee beans, leaving behind carbohydrates, lipids, proteins, metabolites and other chemical compounds. The solid residue left after the brewing process is called spent coffee grounds (SCG). Composition of SCG are commonly found to be around the following values: carbohydrates 39-55% (w/w), lignin 0-26%, lipids 2-24%, proteins 10-18%, chlorogenic acids 1-3%, caffeine 0-0.4%.

SCG were initially considered a waste to be disposed in landfills. This would incur in disposal costs which is not an ideal situation mainly when the so called “waste” has the potential to be converted into value added products. The idea that SCG were waste changed with time and the first applications included its use as animal feed, fertiliser and substrate in the production of fungus. Later, SCG were used in industrial boilers for energy generation. However, due to SCG composition in a wide range of biomolecules has driven research to focus on the use of SCG as a feedstock in the production of value-added products in biorefineries. An advantage of this feedstock is its high content in carbohydrates and relatively high in triglycerides. Additionally, SCG can currently be obtained for free or negligible costs, including its collection and transportation from coffee shops. Currently no research has demonstrated an integrated biorefinery from SCG.

1.5.2.4 Plant/herbaceous based biomass grown specifically for the bioeconomy

A further feedstock for integrated biorefineries are grasses and related plants grown specifically for use in the bioeconomy. Most of the publications presented in this section

focus on the use of grass-based biomass in their integrated biorefinery designs. However, interestingly none of the studies presented actually used the same species of grass. The wide range of available grass-based feedstocks in the different regions of the planet allows for different products to be obtained from different feedstocks and biorefinery designs.

Bals et al presented an integrated biorefinery designed for the extraction of sugars and protein from switchgrass after an ammonia fibre expansion process that was used as a pretreatment (181). This study evaluated the benefits of the protein extraction happening before or after the hydrolysis process. The authors showed that even though the extraction of protein performed previous to the hydrolysis may lead to slightly lower sugar yields than when the extraction was performed after hydrolysis, the trade-off was worthwhile. This is because the extraction of protein after the hydrolysis leads to a much lower protein extraction yield. The protein stream was filtered and the proteins were recovered from this stream, while the sugar stream can be fed into a fermentation process (not performed in this work). The remaining biomass after protein extraction and sugar removal is also ash free. This is because the ash is removed upon protein extraction. Which makes this a good stream for heat and power production.

In 2014 Sun et al have also performed a pretreatment on sweet sorghum stems (182). The study included the hydrolysis of this feedstock to produce alkali-soluble hemicelluloses. The sorghum stems were initially submitted to a hydrothermal pretreatment. Different temperatures (110 to 230 °C) and residence times (30 minutes to 2 hours) were evaluated in this process. The effluent from this process was secondly treated in an alkaline post treatment at 90 °C for 2 hours using a sodium hydroxide concentration of 2% w/w. The results showed that the highest hemicellulose yield of 61% was obtained for a reaction at 130 °C for one hour. In the same year, Sun et al presented a second study with sweet sorghum stems in an identical biorefinery approach to produce xylooligosaccharides and lignin (183). With this study the authors intend to improve the understanding of the lignin hydrolysis allowing its use in biorefineries towards the production of high-value products.

Wu et al used the biomass from *Eulaliopsis binata* to produce dissolving pulp in an integrated biorefinery (184). In this approach the feedstock was initially processed in an acid pretreatment to which removes 90% of the hemicelluloses. The resulting stream was then submitted to an ammonium sulphite treatment followed by potassium hydroxide treatment resulting in a lignin removal of 92%. The resulting pulp showed a α-cellulose purity of 92%, while the pretreatment liquor was rich in xylose. Although the authors did

not perform any more reactions on the liquors obtained, it is mentioned that the pretreatment liquor can be further used in the production of bioethanol by fermentation due to its high xylose content. The authors also suggest that the ammonium sulphite and potassium hydroxide treatment liquor can be used to produce fertiliser.

Popy et al also produced a pulp stream in their study where *Saccharum spontaneum* was used as the feedstock (185). The biomass was pretreated with potassium hydroxide at 90 °C for 2 hours to extract silica, lignin and hemicellulose. This resulted in a lignin, hemicellulose and silica removal of 76%, 41% and 75%, respectively. The resulting pulp was further treated for improved delignification with different concentrations of potassium hydroxide (6 to 12% w/w), different temperatures (70 to 90 °C) and residence times (1 to 2 hours). Lignin was recovered from the black liquor obtained and further upgraded in a four-step synthesis with lignin, phenol, formaldehyde, sodium hydroxide and urea into a lignin-phenol-formaldehyde resin.

Kuglarz et al demonstrated the production of bioethanol and succinic acid in an integrated biorefinery approach using industrial hemp, also known as *Cannabis sativa L.*, as feedstock (186). The hemp was pretreated in two different ways: a) acid pretreatment with 1.5% sulphuric acid by steam injection in the reactor for 10 minutes and b) alkali pretreatment with 3% hydrogen peroxide for a period of 60 to 120 minutes at 90 °C. The acid pretreatment resulted in a high cellulose recovery (higher than 95%) while solubilising 49 to 59% of hemicellulose. After the pretreatment, the effluent was separated into a solid and a liquid fraction. The solid fraction, rich in cellulose, was hydrolysed using enzymes for glucose production and then fermented with *Saccharomyces cerevisiae* into bioethanol. The liquid fraction, mainly rich in xylose, was used in a fermentation with *Actinobacillus succinogenes* to produce succinic acid. It was determined that the alkali pretreatment showed a delignification of 35 to 41% and showed better ethanol yields after enzymatic hydrolysis and fermentation. However, when balancing the ethanol and succinic acid production, the acid hydrolysis showed better results.

Calvo et al demonstrated an integrated biorefinery using *Arundo donax* for the production of polyhydroxyalkanoates (187). The feedstock was initially pre-treated with an ionic liquid (1-ethyl-3-methylidazolium acetate) for 3 hours at a temperature of 160 °C. This was followed by an enzymatic pretreatment using cellulase and hemicellulase to hydrolyse the carbohydrates at 50 °C. The produced fermentable sugars were then used in a dark fermentation process to produce a hydrogen and carbon dioxide-rich gas fraction. No methane production was observed during the experiments. In addition to this

gaseous fraction, the dark fermentation conditions were also designed to produce a liquid fraction rich in organic acids. These organic acids are to be used as substrate in the production of the polyhydroxyalkanoates by biological conversion using bacterial agents. Results show that this operational unit yielded a high concentration of the monomer 3-hydroxybutyrate (95%).

Schwarz et al demonstrated an integrated biorefinery approach that used grass silage to produce fermentable sugars, lignin, steam and electricity (188). The grass silage was initially separated into a liquid and a solid phase. The liquid phase denominated as press juice was directed into a fermentation, while the cake was submitted to a mechanical saccharification. The effluent from this saccharification was fed into a second solid-liquid separation. The hydrolysate could be used in a fermentation process while the solid phase was further processed in an organosolvation which consisted of adding a solution of ethanol and water in a ratio of 60-40 to the solids and heat this mixture until 195 °C for 80 minutes. The products from this process were separated in a third solid-liquid separation. Lignin was also recovered from the liquid fraction by precipitation. The authors suggest that resulting stream can be used in an anaerobic digestion for heat and power generation. Finally, the solid fraction was further hydrolysed. In a later study in 2018, Schwarz et al built on their previous work to demonstrated that grass silage can also be used to produce poly-3-hydroxybutyrate (189). In this approach the feedstock was separated in a press juice and a press cake. The press juice was used as a nutrient source for the growth of *Cupriavidus necator* while the press cake was used in a mechanically assisted saccharification. The hydrolysates were separated from the solids and used in a lactic acid fermentation. This broth was then combined with the full-grown cell of *Cupravidus necator* to produce poly-3-hydroxybutyrate which was further purified.

Other research has focused on the use of seeds as feedstocks in biorefinery approaches. Kurniawan et al used kapok seeds in an integrated biorefinery study to converted them into biofuels (190). The seeds and the seed cake were initially submitted to a noncatalytic subcritical methanol transesterification process using methanol produced in situ to yield biodiesel. The remaining biomass after the transesterification process was fed into a pyrolysis unit to produce bio-oil and biochar. Indian mustard seed oil plant was used by Chen et al to produce ethyl biodiesel and bio-lubricants (191). While the ethyl biodiesel was produced by ethanolysis at 35 °C for 50 minutes and a potassium hydroxide content of 1.1% w/w. This unpurified ethyl biodiesel was then used in the production of bio-lubricant.

This further demonstrates that second generation biomass can be processed in an integrated fashion using multiple unit operations to produce an array of products with little waste generated.

1.5.2.5 Future sources of biomass: 3rd generation marine biomass

While the use of second generation lignocellulose discussed above is well established, concerns over competition with land, the ultimate need for higher yields and competition with virgin forest have led to a large research effort in alternative marine feedstocks. In this section, a group of innovative biomass sources are presented as possible future feedstocks in the production of biofuels in biorefineries. All work done to date on these resources is still on the lab or pilot scale but shows promise to deliver another biomass resource for the bioeconomy.

Microalgae

Microalgae consist of a varied set of prokaryotic and eukaryotic unicellular microorganisms that can be grown in adverse conditions due to their simple cellular structure. These microorganisms can be found both in terrestrial and aquatic environments. The latter include both freshwater and saltwater ecosystems. The wide variety of strains that can be found in all of these ecosystems account for thousands of species. Mata et al reported that there are around 50 thousand microalgae species, from which only 60% have been studied (192).

Microalgae were firstly grown in commercially large-scale systems back in the 1960's when researchers in Japan efficiently grown a culture of *Chlorella* (193,194). This was followed by a growth of *Arthrospira platensis* in the 1970's in Mexico (194). In fact, the interest in the production of energy from microalgae sources grew in the 1970's due to the first oil crisis (193). This started some research programs such as the one at the U.S. National Renewable Energy Laboratory which initially started on the production of hydrogen from microalgae but later focused on the production of biodiesel from the same feedstock (195). Researchers concluded that this was a feasible route, however it requires further research due to the high costs registered both in the microalgae growth and its upgrading to biodiesel. Current worldwide problems such as climate change and global warming, in combination with the crude oil price fluctuations, have increased the recent interest in renewable and greener solutions such as microalgae towards the production of biofuels. Microalgae are still heavily researched for biofuels production which is shown by the increasing number of studies using this biomass in biorefineries.

These microorganisms can grow in very harsh conditions due to their adaptability and ability to change their internal structure (192). Another microalgae advantage is their

ability to grow using different metabolisms, which can go from autotrophic, heterotrophic, mixotrophic to photoheterotrophic. The cultivation of microalgae requires mainly a source of carbon, nutrients and light to subsist and perform its photosynthesis activity (196). Other parameters include temperature, pH, amount and type of nutrients supplied.

The cultivation of microalgae in biorefineries and in the production of biofuels requires a previous evaluation on the type of microalgae and its conditions of growth. This is not only due to a correct growth of the microalgae but also its appropriate content of its constituents. As an example, a biorefinery primarily designed for the production of biodiesel will preferably use a microalga with higher content on lipids, while a bioethanol biorefinery will focus on microalgae with higher content in carbohydrates. Additionally, the harvest time also has an influence on the microalgae content. On their study on the influence of the harvest time on the conversion yield, Dong et al have also reported a high protein content for early harvest microalgae, higher carbohydrate content for mid and late-harvest time and high lipid content for late harvest time (197).

Microalgae present some important properties that contribute to its increased popularity in the production of biofuels. Some of these advantages include the ability of microalgae to grow in water unsuitable for human consumption, high growth rates and high overall biomass yields when compared to forestry and agricultural biomass (192). However, there are still many challenges to overcome. The processing of the microalgae needs consider its water content. If a dried biomass is required, a dewatering step is needed which increases the biofuel production costs (198). The lipid extraction and purification processes are also highly energy intensive (199). Microalgae are seasonal microorganisms which leads to a lower availability of this feedstock in certain seasons throughout the year. These reasons lead to a biofuel production process with increased costs. To make microalgae-based biofuels competitive with fossil fuels, further research is needed.

Subhash and Mohan were amongst the first authors to present a microalgae based biorefinery in 2014 when they demonstrated the production of biohydrogen from a fat-free microalgae mixture (after lipid extraction) (200). In this study the fat-free microalgae were initially acid pretreated for 30 minutes with 3% of sulphuric acid at 121 °C. The pretreated effluent was separated into a solid and liquid fraction by centrifugation. The separated solid, separated liquid and an unseparated fraction of the pretreated effluent were all submitted to separate dark fermentations for biohydrogen production. The results show that the separated liquid fraction resulted in a higher biohydrogen yield (217

ml per grams of algal biomass) when compared to the separated solid and the unseparated pretreated effluent (122 and 112 ml/g of algal biomass, respectively).

In 2015 Patnaik and Mallick developed an integrated biorefinery that used the microalgae *Scenedesmus obliquus* to produce biodiesel, glycerol, omega-3 fatty acids and bioethanol (201). The first step in this biorefinery approach is the lipid extraction using a mixture of chloroform and methanol as extraction solvent in the ratio of 1:2. These lipids were then transesterified, yielding 38 g of biodiesel from 100 of microalgae. 3 g of glycerol and 2 g of omega-3 fatty acids were obtained as by-products from the transesterification of lipids. The free-lipid biomass was then used in an acid hydrolysis with 2 N of sulphuric acid to obtain fermentable sugars that were fermented with *Saccharomyces cerevisiae* to obtain 17 g of bioethanol from 100 g of biomass.

A specie of photosynthetic cyanobacterium, *Arthrospira platensis* (Spirulina), was used in an integrated biorefinery study towards the production of bioethanol and lactic acid in the work presented by Esquivel-Hernandez et al in 2019 (202). In this investigation three different pretreatments for metabolites extraction were studied: supercritical fluid extraction, microwave assisted extraction with polar solvents and with non-polar solvents. The pretreated biomass was then fermented with *Saccharomyces cerevisiae* and *Lactobacillus acidophilus* to yield bioethanol and lactic acid, respectively. The microwave assisted extraction with non-polar solvents resulted in the highest bioethanol concentration achieved with 3.0 g/L, while the supercritical fluid extraction yielded the highest concentration of lactic acid obtained, 9.7 g/L.

Later in 2016 Dong et al also used a strain from the genus *Scenedesmus*, *Scenedesmus acutus,* to compare two different integrated biorefinery approaches for the production of bioethanol and lipids (203). In the first approach the microalga was fed into an acid pretreatment at 155 °C for 15 minutes with an acid concentration of 2% (w/w). This pretreatment acts as a hydrolysis step for the subsequent processes. The resulting stream was used in a solid-liquid separation where the liquor is fed into a fermentation with *Saccharomyces cerevisiae* to yield bioethanol. The bioethanol was recovered from the fermentation broth by distillation. The solid obtained in the solid-liquid separation was submitted to a lipid extraction. The authors concluded that the lipids were mainly entrapped in the solids. Therefore, the lipids were extracted from the solids using hexane as extraction solvent. The hexane was then recovered by distillation and recycled to the lipid extraction while the lipids were collected after distillation. However, the authors also determined that in this approach there is a high concentration of carbohydrates entrapped in the solid fraction. Therefore, a new approach was developed. In this

subsequent approach, the microalgae were submitted to the same acid pretreatment. The major change was there was no solid-liquid separation as the pretreated effluent was directly used in the fermentation (same conditions as before). The fermented broth was then distilled for ethanol recovery while the remaining fermented broth was used in lipid extraction and distillation for lipid recovery. The authors concluded that in this second approach almost all the fermentable sugars were used in the production of bioethanol. In fact, this approach led to an improved utilisation of the carbohydrates to bioethanol, 31 GGE per ton of biomass compared to the 20 GGE per ton of biomass obtained in the initial approach. It was also concluded that the fermentation of the pretreated slurry did not affect the lipids in solution. An 87% of lipid recovery was achieved in this approach. The recovered lipids can be further used in the production of biodiesel or in the production of non-isocyanate polyurethanes due to the high content of unsaturated fatty acids in the lipid fraction.

In 2018 Zhang et al presented an integrated biorefinery based on another microalgae type, *Phaeodactylum tricornutum* (204). This approach used a series of extractions and purifications to obtain three bioactive compounds. The microalga was initially used in an ethanol extraction to yield an ethanol extract and residue 1. Both of these intermediates were then submitted to a hexane extraction separately from where both hexane phases obtained were combined, concentrated and purified to obtain a concentrated eicosapentaenoic acid. The other stream obtained from the hexane extraction of the ethanol extract was called a hydroalcoholic phase that was purified to obtain fucoxanthin. Finally, the other product of the hexane extraction of the residue 1 is called residue 2. This is submitted to deproteinization and further purified to obtain a purified stream of chrysolaminarin.

In 2020 the seasonality of microalgae (*Monoraphidium sp.*) was addressed by blending it with domestic sewage water sludge (205). The blends of these two feedstocks were processed in a hydrothermal liquefaction process. The authors studied variables such as feedstock ratio, reaction temperature and duration to optimise the biocrude production. The results show the optimal conditions include a 3:1 ratio of microalgae to domestic sewage sludge, 325 °C for a period of 45 minutes. A yield of 39.4% (w/w) was obtained for the abovementioned conditions. This yield was higher than the one achieved when processing this microalga and the domestic sewage sludge separately.

Ashokkumar et al have also used domestic sewage but this time to grow *Chlorella sp.* and used the grown microalgae in the production of biodiesel and biochar (206). In this approach a Soxhlet extraction method with chloroform and methanol in a ratio of 2:1 was

used to extract the lipids from *Chlorella sp.* The lipids were then separated from the extraction solvents by distillation and converted to biodiesel in a two -step method: esterification of the free fatty acids followed by transesterification to biodiesel. The lipid-free biomass was further used in the production of biochar by slow pyrolysis at 400 °C.

Macroalgae and aquatic plants

Macroalgae have a much more complex structure when compared to microalgae. While microalgae are prokaryotic and singular eukaryotic microorganisms, macroalgae have multicellular eukaryotic organisms. Macroalgae sizes can go from a few millimetres to a length up to 50 metres. Macroalgae can be classified into three main types *Heterokontophyta* or *Ochrophyta* (brown algae), *Chlorophyta* (green algae) and *Rhodophyta* (red algae) (207). This classification depends on the pigments present in these macroalgae, apart from *chlorophyll* that is present in all these classes.

Macroalgae was initially used for food and medical purposes. However, due to the current climate change crisis and all the implications associated to it, researchers started evaluating macroalgae as a feedstock towards the production of biofuels. The Food and Agriculture Organization of the United Nations reported an increasing production of seaweed worldwide every year. Additionally, out of the 32.4 million tonnes of seaweed collected in 2018, 97.1% were farmed (208). These numbers demonstrate the increased attention that has been payed to seaweed. Even though most of these macroalgae are for food purposes, there is the potential, technology, knowledge and space availability for the production of macroalgae. In fact, the production of macroalgae presents some relevant advantages such as the availability of unused ocean areas, seaweed are a high carbohydrate and low lignin biomass source, no need of freshwater for its growth, carbon dioxide fixation, fast and straightforward growth.

Naturally, the numerous macroalgae across the planet have different compositions due to their different species and environments that they grew in. Due to this variability, it is difficult to quantify the biochemical composition of macroalgae. Several authors assessed some macroalgae species commonly found in their regions. Examples include authors such as Beacham et al, Parthiban et al, Rohani-Ghadikolaei et al and Manivannan et al have presented studies that include seaweed biochemical composition in South-West England, Tuticorin coast in Malaysia, Persian Gulf of Iran and southeast cost of India, respectively (209–212).

Macroalgae has also been studied as a possible feedstock in the production of biofuels. In 2020 Prabhu et al demonstrated a biorefinery approach to process a strain of *Ulva ohnoi*, in a series of filtration and extraction steps to yield several products (213). The

authors used a combination of filtration and extraction steps to yield a salt-rich fraction, a starch-rich fraction, a lipid-rich fraction, an Ulvan-rich fraction, a protein-rich fraction and a cellulose rich-fraction. The salt and starch-rich fraction are obtained from the microfiltration of the homogenised feedstock in water. The filtrate was centrifuged to yield a liquid phase from where the salt-rich fraction was obtained and a solid fraction to be washed with ethanol and centrifuged again to obtain a solid starch fraction. The solid fraction obtained from microfiltration was washed with ethanol previous to a second microfiltration. This second filtrate was concentrated for lipid recovery while the solid fraction was submitted to centrifugation for ulvan-rich fraction removal. The solid resultant from this process was finally submitted to another extraction and centrifugation to yield a liquid fraction rich in protein and a solid fraction rich in cellulose.

Azolla filiculoides, a strain of an aquatic fern, has also been assessed as a feedstock in the biorefinery presented by Dohaei et al in 2020 (214). Just like in the study presented by Esquivel-Hernandez et al, the presented study also combines a series of extraction and purification processes but this time to recover lipids, protein and phenolics from the biomass, previous to the production of biomethane. The feedstock was initially used in a lipid extraction using a Soxhlet method with hexane for 24 hours, from where a maximum lipid yield of 18% was achieved. The resulting biomass fraction was then submitted to a protein extraction using two extraction steps. The first extraction was performed using an ultrasound assisted water method with sodium hydroxide extraction to achieve a 24% recovery of protein. The second extraction used trichloroacetic acid which resulted in a 12% protein recovery. The resulting biomass was then submitted to an extraction of the phenolic compounds from where chlorogenic acid, caffeic acid and tannic acid were obtained. Finally, the solid stream obtained after this series of extractions was used in an anaerobic digestion to produce biomethane.

1.6 Overall products from integrated biorefineries

All of these examples of integrated biorefineries produce a wide range of products. With over eighteen different products appearing in the reviewed literature more than one time (Figure 1-5). The products that only appeared once in the literature were grouped and are represented in the last column called “Others”. Bioethanol and carbohydrates appear 30 and 24 times, respectively in the literature. The production of carbohydrates in reviewed studies were most commonly applied to produce bioethanol by fermentation. Even when this fermentation step was not completed many of the authors that achieved the production of carbohydrates as a final product, mentioned bioethanol as a possible application. Considering the abovementioned, there is a trend in the literature towards the production of bioethanol in integrated biorefinery approaches. This might be due to the straightforward and low-cost fermentation process of the fermentable sugars into bioethanol. Also, there are already circulating in the market blendstocks of petrol products and bioethanol that aim to help in the transition towards more sustainable use of fuels in the transportation sector.

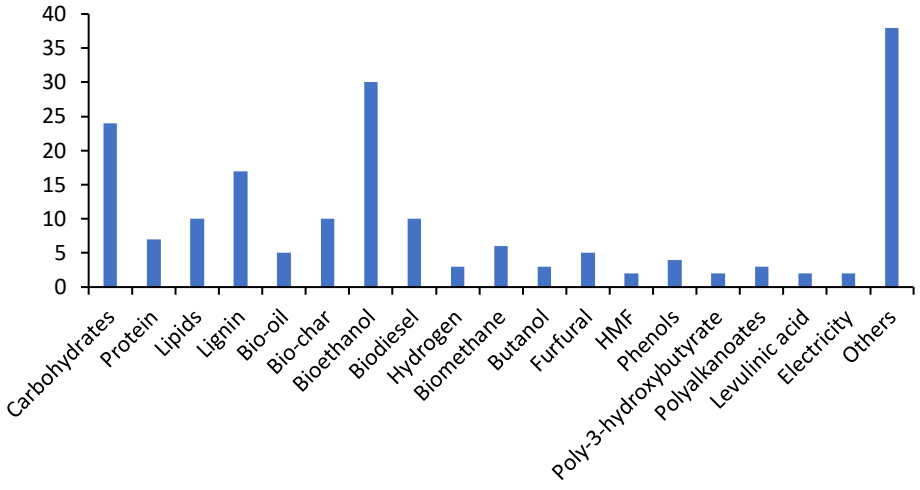

Figure 1-5 – Number of times the products appeared in the literature including “integrated biorefinery” as product or by-product

Lipids and biodiesel are among the most commonly obtained products in the literature with at least 10 applications. Just like in the previous case, lipids are the feedstock used in the production of biodiesel by a transesterification process. These results show the importance of biodiesel has in the research community as one of the main biofuels to replace petrol-based fuels, diesel in this case.

Other products with high significance in the reviewed literature include protein (7), lignin (17), bio-oil (5), biochar (10) and biomethane (6). Just like in the case of carbohydrates with bioethanol and lipids with biodiesel, lignin, bio-oil and biochar can also be considered feedstock-product pairs. An attractive application of lignin is its use in pyrolysis and hydrothermal liquefaction processes to yield both bio-oil and biochar. Even though lignin contains a wide range of phenolic compounds that can be further employed in the production of high-value product, its recalcitrant nature makes it difficult to be broken into its monomers (215). However, advances have been made and recent research demonstrated effective methods to depolymerise lignin into its monomers which allows the conversion of these monomers into high-value products (216,217).

1.7 Aims and Objectives

The overall aim of this thesis is to develop an integrated biorefinery around the use of spent coffee grounds (SCG). While a number of studies have demonstrated the suitability of coffee grounds for valorisation, this is yet to be established in an integrated biorefinery methodology, where all fractions of the biomass are valorised and multiple feedstocks can be appropriately used.

To this end the first objective, detailed in the first research chapter is to assess SCG as a feedstock alone, with the aim of producing 5-hydroxymethylfurfural (HMF), lipids and a solid heating fuel. A simple organosolv fractionation will be assessed and the value compounds produced alongside this product established.

The second objective, given in the second research chapter, is to determine the possibility of using SCG co-processed with the lipid rich microalgae *Scenedesmus acutus.* This will establish whether SCG could be used to tackle the low availability of microalgae during winter and autumn seasons. The feedstocks (SCG and microalgae) will be processed separately as a control and together in two different percentage blends to simulate these seasons. The aim of this section will be to establish the suitability for producing lipids, ethanol through fermentation and HTL products.

The final objective, given in the final research chapter will assess the production of 5-hydroxymethylfurfural in a biorefinery, though using alternative blends of macroalgae and SCG. As in the previous chapter, SCG will be used to simulate making up for the low availability of seaweed in short-supply seasons, with HMF, lipids and HTL products all being assessed to valorise the full feedstock.

1.8 References

1. Oil left in the word. 2020.
2. de Jong E, van Ree R, Kwant IK. Biorefineries: Adding value to the sustainable utilisation of biomass. IEA Bioenergy. 2009.
3. Agbor VB, Cicek N, Sparling R, Berlin A, Levin DB. Biomass pretreatment: Fundamentals toward application. Biotechnol Adv. 2011;29(6):675–85.
4. Silva GGD, Couturier M, Berrin J-G, Buléon A, Rouau X. Effects of grinding processes on enzymatic degradation of wheat straw. Bioresour Technol. 2012;103(1):192–200.
5. Mayer-Laigle C, Blanc N, Rajaonarivony RK, Rouau X. Comminution of Dry Lignocellulosic Biomass, a Review: Part I. From Fundamental Mechanisms to Milling Behaviour. Bioeng. 2018;5(2):41-54.
6. Pielhop T, Amgarten J, von Rohr PR, Studer MH. Steam explosion pretreatment of softwood: the effect of the explosive decompression on enzymatic digestibility. Biotechnol Biofuels. 2016;9:152-64.
7. Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M, Ladisch M. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour Technol. 2005;96(6):673–86.
8. Li J, Henriksson G, Gellerstedt G. Lignin depolymerization/repolymerization and its critical role for delignification of aspen wood by steam explosion. Bioresour Technol. 2007;98(16):3061–8.
9. Schultz TP, Biermann CJ, McGinnis GD. Steam explosion of mixed hardwood chips as a biomass pretreatment. Ind Eng Chem Prod Res Dev. American Chemical Society. 1983;22(2):344–8.
10. Nguyen LM. Organic matter composition, microbial biomass and microbial activity in gravel-bed constructed wetlands treating farm dairy wastewaters. Ecol Eng. 2000;16(2):199–221.
11. Zhao X, Cheng K, Liu D. Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis. Appl Microbiol Biotechnol. 2009;82(5):815-827.
12. Wildschut J, Smit AT, Reith JH, Huijgen WJJ. Ethanol-based organosolv fractionation of wheat straw for the production of lignin and enzymatically digestible cellulose. Bioresour Technol. 2013;135:58–66.

13. Brudecki G, Cybulska I, Rosentrater K. Optimization of clean fractionation process applied to switchgrass to produce pulp for enzymatic hydrolysis. Bioresour Technol. 2013;131:101–12.

14. Bozell JJ, O'Lenick CJ, Warwick S. Biomass fractionation for the biorefinery: heteronuclear multiple quantum coherence-nuclear magnetic resonance investigation of lignin isolated from solvent fractionation of switchgrass. J Agric Food Chem. 2011;59(17):9232–42.

15. Zhang Y-HP, Ding S-Y, Mielenz JR, Cui J-B, Elander RT, Laser M, Himmel ME, McMillan JR, Lynd LR. Fractionating recalcitrant lignocellulose at modest reaction conditions. Biotechnol Bioeng. 2007;97(2):214–23.

16. Pan X, Arato C, Gilkes N, Gregg D, Mabee W, Pye K, Xiao Z, Zhang X, Saddler J. Biorefining of softwoods using ethanol organosolv pulping: Preliminary evaluation of process streams for manufacture of fuel-grade ethanol and co-products. Biotechnol Bioeng. 2005;90(4):473–81.

17. Bozell JJ, Black SK, Myers M, Cahill D, Miller WP, Park S. Solvent fractionation of renewable woody feedstocks: Organosolv generation of biorefinery process streams for the production of biobased chemicals. Biomass and Bioenergy. 2011;35(10):4197–208.

18. Black SK, Hames BR, Myers MD. Method of separating lignocellulosic material into lignin, cellulose and dissolved sugars. 1998.

19. Katahira R, Mittal A, McKinney K, Ciesielski PN, Donohoe BS, Black SK, Johnson DK, Biddy MJ, Beckham GT. Evaluation of Clean Fractionation Pretreatment for the Production of Renewable Fuels and Chemicals from Corn Stover. ACS Sustain Chem Eng. 2014;2(6):1364–76.

20. Schutyser W, Renders T, den Bosch S, Koelewijn S-F, Beckham GT, Sels BF. Chemicals from lignin: an interplay of lignocellulose fractionation, depolymerisation, and upgrading. Chem Soc Rev.2018;47(3):852–908.

21. Zhang Z, Harrison MD, Rackemann DW, Doherty WOS, O'Hara IM. Organosolv pretreatment of plant biomass for enhanced enzymatic saccharification. Green Chem. 2016;18(2):360–81.

22. Smit A, Huijgen W. Effective fractionation of lignocellulose in herbaceous biomass and hardwood using a mild acetone organosolv process. Green Chem. 2017;19(22):5505–14.

23. Resch MG, Donohoe BS, Ciesielski PN, Nill JE, Magnusson L, Himmel ME, Mittal A, Katahira R, Biddy MJ, Beckham GT. Clean Fractionation Pretreatment Reduces Enzyme Loadings for Biomass Saccharification and Reveals the Mechanism of Free and Cellulosomal Enzyme Synergy. ACS Sustain Chem Eng. 2014;2(6):1377–87.

24. Teng J, Ma H, Wang F, Wang L, Li X. Catalytic Fractionation of Raw Biomass to Biochemicals and Organosolv Lignin in a Methyl Isobutyl Ketone/H2O Biphasic System. ACS Sustain Chem Eng. 2016;4(4):2020–6.

25. Stiefel S, Di Marino D, Eggert A, Kühnrich IR, Schmidt M, Grande PM, Leitner W, Jupke A, Wessling M. Liquid/liquid extraction of biomass-derived lignin from lignocellulosic pretreatments. Green Chem. 2017;19(1):93–7.

26. Mittal A, Vinzant TB, Brunecky R, Black SK, Pilath HM, Himmel ME, Johnson DK. Investigation of the role of lignin in biphasic xylan hydrolysis during dilute acid and organosolv pretreatment of corn stover. Green Chem. 2015;17(3):1546–58.

27. Zimmermann CJ, Bollar N V, Wettstein SG. Liquid phase conversion of lignocellulosic biomass using biphasic systems. Biomass and Bioenergy. 2018;118:163–71.

28. Suriyachai N, Champreda V, Kraikul N, Techanan W, Laosiripojana N. Fractionation of lignocellulosic biopolymers from sugarcane bagasse using formic acid-catalyzed organosolv process. 3 Biotech. 2018;8(5):221.

29. Sindhu R, Binod P, Pandey A. Biological pretreatment of lignocellulosic biomass – An overview. Bioresour Technol. 2016;199:76–82.

30. Zabed HM, Akter S, Yun J, Zhang G, Awad FN, Qi X, Sahu JN. Recent advances in biological pretreatment of microalgae and lignocellulosic biomass for biofuel production. Renew Sustain Energy Rev. 2019;105:105–28.

31. Demirbas A. Combustion characteristics of different biomass fuels. Prog Energy Combust Sci. 2004;30(2):219–30.

32. Dry ME. The Fischer–Tropsch process: 1950–2000. Catal Today. 2002;71(3):227–41.

33. Abatzoglou N, Dalai A, Gitzhofer F. Green Diesel from Fischer-Tropsch Synthesis: Challenges and Hurdles. Proc of the 3rd IASME/WSEAS Int Conf on Energy, Environment, Ecosystems and Sustainable Development. 2007; 223–32.

34. Choudhury HA, Chakma S, Moholkar VS. Chapter 14 - Biomass Gasification Integrated Fischer-Tropsch Synthesis: Perspectives, Opportunities and Challenges. Pandey A, Bhaskar T, Stöcker M, Sukumaran RKBT-RA in T-CC of B. 2015; 383–435.

35. Demirbaş A. Mechanisms of liquefaction and pyrolysis reactions of biomass. Energy Convers Manag. 2000;41(6):633–46.

36. Lu X, Yamauchi K, Phaiboonsilpa N, Saka S. Two-step hydrolysis of Japanese beech as treated by semi-flow hot-compressed water. J Wood Sci. 2009;55(5):367–75.

37. Dimitriadis A, Bezergianni S. Hydrothermal liquefaction of various biomass and waste feedstocks for biocrude production: A state of the art review. Renew Sustain Energy Rev. 2017;68:113–25.

38. Sevilla M, Fuertes AB. The production of carbon materials by hydrothermal carbonization of cellulose. Carbon N Y. 2009;47(9):2281–9.

39. Krylova AY, Zaitchenko VM. Hydrothermal Carbonization of Biomass: A Review. Solid Fuel Chem. 2018;52(2):91–103.

40. Jiang W, Kumar A, Adamopoulos S. Liquefaction of lignocellulosic materials and its applications in wood adhesives—A review. Ind Crops Prod. 2018;124:325–42.

41. Bensaid S, Conti R, Fino D. Direct liquefaction of ligno-cellulosic residues for liquid fuel production. Fuel. 2012;94:324–32.

42. Zhu Z, Rosendahl L, Toor SS, Yu D, Chen G. Hydrothermal liquefaction of barley straw to bio-crude oil: Effects of reaction temperature and aqueous phase recirculation. Appl Energy. 2015;137:183–92.

43. Beims RF, Hu Y, Shui H, Xu C (Charles). Hydrothermal liquefaction of biomass to fuels and value-added chemicals: Products applications and challenges to develop large-scale operations. Biomass and Bioenergy. 2020;135:105510.

44. Trivedi J, Aila M, Bangwal DP, Kaul S, Garg MO. Algae based biorefinery—How to make sense? Renew Sustain Energy Rev. 2015;47:295–307.

45. Biller P, Ross AB, Skill SC, Lea-Langton A, Balasundaram B, Hall C, Riley R, Llewellyn CA. Nutrient recycling of aqueous phase for microalgae cultivation from the hydrothermal liquefaction process. Algal Res. 2012;1(1):70–6.

46. Furusawa T, Sato T, Sugito H, Miura Y, Ishiyama Y, Sato M, Itoh N, Suzuki N.

Hydrogen production from the gasification of lignin with nickel catalysts in supercritical water. Int J Hydrogen Energy. 2007;32(6):699–704.

47. Usman M, Hao S, Chen H, Ren S, Tsang DCW, O-Thong S, Luo G, Zhang S. Molecular and microbial insights towards understanding the anaerobic digestion of the wastewater from hydrothermal liquefaction of sewage sludge facilitated by granular activated carbon (GAC). Environ Int. 2019;133:105257.

48. Ahmad F, Silva EL, Varesche MBA. Hydrothermal processing of biomass for anaerobic digestion – A review. Renew Sustain Energy Rev. 2018;98:108–24.

49. Taherzadeh MJ, Karimi K. Enzyme-based hydrolysis processes for ethanol from lignocellulosic materials: a review. BioResources. 2007;2:707-38.

50. Ogier JC, Leygue JP, Ballerini D, Rigal L, Pourquie J. Ethanol production from lignocellulosic biomass; Production d`ethanol a partir de biomasse lignocellulosique. Rev l'Institute Fr du Pet. 1999;54(1):67-94.

51. Lee YY, Iyer P, Torget RW. Dilute-acid hydrolysis of lignocellulosic biomass. Recent progress in bioconversion of lignocellulosics. 1999. 93–115.

52. Taherzadeh MJ. Ethanol from lignocellulose: physiological effects of inhibitors and fermentation strategies. 1999;1485:1-57.

53. Yu IKM, Chen H, Abeln F, Auta H, Fan J, Budarin VL, Clark JH, Parsons S, Chuck CJ, Zhang S, Luo G, Tsang DCW. Chemicals from lignocellulosic biomass: A critical comparison between biochemical, microwave and thermochemical conversion methods. Crit Rev Environ Sci Technol. 2020; 1–54.

54. Stanbury PF, Whitaker A, Hall SJ. Principles of Fermentation Technology. 3rd Editio. 2016.

55. Abeln F, Fan J, Budarin VL, Briers H, Parsons S, Allen MJ, Henk DA, Clark J, Chuck CJ. Lipid production through the single-step microwave hydrolysis of macroalgae using the oleaginous yeast Metschnikowia pulcherrima. Algal Res. 2019;38:1-9.

56. Ward AJ, Lewis DM, Green FB. Anaerobic digestion of algae biomass: A review. Algal Res. 2014;5:204–14.

57. Chynoweth DP, Owens JM, Legrand R. Renewable methane from anaerobic digestion of biomass. Renew Energy. 2001;22(1):1–8.

58. McCarty PL. Anaerobic Waste Treatment Fundamentals. Chem Biol.

1964;95(9):123–6.

59. Pileidis FD, Titirici M-M. Levulinic Acid Biorefineries: New Challenges for Efficient Utilization of Biomass. Chem Sus Chem. 2016;9(6):562–82.

60. Saha B, Abu-Omar MM. Advances in 5-hydroxymethylfurfural production from biomass in biphasic solvents. Green Chem. 2014;16(1):24–38.

61. Hayes DJ, Fitzpatrick S, Hayes MHB, Ross JRH. The Biofine Process – Production of Levulinic Acid, Furfural, and Formic Acid from Lignocellulosic Feedstocks. Biorefineries-Industrial Processes and Products. 2008;1:139–64.

62. Werpy T, Petersen G. Top Value Added Chemicals from Biomass: Volume I -- Results of Screening for Potential Candidates from Sugars and Synthesis Gas. 2004.

63. Mathimani T, Uma L, Prabaharan D. Homogeneous acid catalysed transesterification of marine microalga Chlorella sp. BDUG 91771 lipid – An efficient biodiesel yield and its characterization. Renew Energy. 2015;81:523–33.

64. Hariram V, John JG, Seralathan S. Spectrometric analysis of algal biodiesel as a fuel derived through base-catalysed transesterification. Int J Ambient Energy. 2019;40(2):195–202.

65. IEA. Bioenergy Power Generation. 2020.

66. IEA. Tracking Power. 2020.

67. Andersen L, Lamp A, Dieckmann C, Baetge S, Schmidt L-M, Kaltschmitt M. Biogas plants as key units of biorefinery concepts: Options and their assessment. J Biotechnol. 2018;283:130–9.

68. Bharathiraja B, Sudharsana T, Jayamuthunagai J, Praveenkumar R, Chozhavendhan S, Iyyappan J. Biogas production - A review on composition, fuel properties, feed stock and principles of anaerobic digestion. Renew Sustain Energy Rev. 2018;90(C):570–82.

69. Weiland P. Biogas production: current state and perspectives. Appl Microbiol Biotechnol. 2010;85(4):849–60.

70. Roubaud A, Roethlisberger RP, Favrat D. Lean-Burn Cogeneration Biogas Engine with Unscavenged Combustion Prechamber: Comparison with Natural Gas. In 2002;5:169-76.

71. Crookes RJ. Comparative bio-fuel performance in internal combustion engines. Biomass and Bioenergy. 2006;30:461–8.

72. Porpatham E, Ramesh A, Nagalingam B. Investigation on the effect of concentration of methane in biogas when used as a fuel for a spark ignition engine. Fuel. 2008;87:1651–9.

73. Preethi, Usman TMM, Rajesh Banu J, Gunasekaran M, Kumar G. Biohydrogen production from industrial wastewater: An overview. Bioresour Technol Reports. 2019;7:100287.

74. Plangklang P, Reungsang A, Pattra S. Enhanced bio-hydrogen production from sugarcane juice by immobilized Clostridium butyricum on sugarcane bagasse. Int J Hydrogen Energy. 2012;37(20):15525–32.

75. Das D, Veziroğlu TN. Hydrogen production by biological processes: a survey of literature. Int J Hydrogen Energy. 2001;26(1):13–28.

76. Argun H, Dao S. Bio-hydrogen production from waste peach pulp by dark fermentation: Effect of inoculum addition. Int J Hydrogen Energy. 2017;42(4):2569–74.

77. Łukajtis R, Hołowacz I, Kucharska K, Glinka M, Rybarczyk P, Przyjazny A, Kaminski M. Hydrogen production from biomass using dark fermentation. Renew Sustain Energy Rev. 2018;91:665–94.

78. Krylova AY, Kozyukov EA, Lapidus AL. Ethanol and diesel fuel from plant raw materials: A review. Solid Fuel Chem. 2008;42(6):358–64.

79. Kasavi C, Finore I, Lama L, Nicolaus B, Oliver SG, Toksoy Oner E, Kirdar B. Evaluation of industrial Saccharomyces cerevisiae strains for ethanol production from biomass. Biomass and Bioenergy. 2012;45:230–8.

80. Lin Y, Zhang W, Li C, Sakakibara K, Tanaka S, Kong H. Factors affecting ethanol fermentation using Saccharomyces cerevisiae BY4742. Biomass and Bioenergy. 2012;47:395–401.

81. Kobayashi Y, Sahara T, Suzuki T, Kamachi S, Matsushika A, Hoshino T, Ohgiya S, Kamagata Y, Fujimori KE. Genetic improvement of xylose metabolism by enhancing the expression of pentose phosphate pathway genes in Saccharomyces cerevisiae IR-2 for high-temperature ethanol production. J Ind Microbiol Biotechnol. 2017;44(6):879–91.

82. Hohenschuh W, Hector RE, Mertens JA, Murthy GS. Development and characterization of Saccharomyces cerevisiae strains genetically modified to over-express the pentose phosphate pathway regulating transcription factor STB5 in the presence of xylose. Syst Microbiol Biomanufacturing. 2020;1(1):42-57

83. European Automobile Manufacturers Association. Joint declaration for legal consistency of the EU alternative fuels definition. 2020.

84. Demirbas A. Biomethanol Production from Organic Waste Materials. Energy Sources, Part A Recover Util Environ Eff. 2008;30(6):565–72.

85. Hasegawa F, Yokoyama S, Imou K. Methanol or ethanol produced from woody biomass: Which is more advantageous? Bioresour Technol. 2010;101(1, Supplement):S109–11.

86. Gautam P, Neha, Upadhyay SN, Dubey SK. Bio-methanol as a renewable fuel from waste biomass: Current trends and future perspective. Fuel. 2020;273:117783.

87. Demirbas MF, Balat M. Biomass pyrolysis for liquid fuels and chemicals: A review. CSIR; 2007;797-804.

88. IEA. Chemicals. 2020.

89. Aho A, Tokarev A, Backman P, Kumar N, Eränen K, Hupa M, et al. Catalytic Pyrolysis of Pine Biomass Over H-Beta Zeolite in a Dual-Fluidized Bed Reactor: Effect of Space Velocity on the Yield and Composition of Pyrolysis Products. Top Catal. 2011;54(13):941.

90. Taarning E, Osmundsen CM, Yang X, Voss B, Andersen SI, Christensen CH. Zeolite-catalyzed biomass conversion to fuels and chemicals. Energy Environ Sci. 2011;4(3):793–804.

91. Hussain Z, Khan KM, Khan A, Ullah S, Karim A, Perveen S. The conversion of biomass into liquid hydrocarbon fuel by two step pyrolysis using cement as catalyst. J Anal Appl Pyrolysis. 2013;101:90–5.

92. Hu X, Gholizadeh M. Progress of the applications of bio-oil. Renew Sustain Energy Rev. 2020;134:110124.

93. EIA. EIA projects US biofuel production to slowly increase through 2050. 2020.

94. Wongwuttanasatian T, Jookjantra K. Effect of dual-frequency pulsed ultrasonic excitation and catalyst size for biodiesel production. Renew Energy.

2020;152:1220–6.

95. Musa IA. The effects of alcohol to oil molar ratios and the type of alcohol on biodiesel production using transesterification process. Egypt J Pet. 2016;25(1):21–31.

96. Gao Y-Y, Chen W-W, Lei H, Liu Y, Lin X, Ruan R. Optimization of Transesterification Conditions for the Production of Fatty Acid Methyl Ester (FAME) from Chinese Tallow Kernel Oil with a Nano-Scale Magnetic Catalyst. Trans ASABE. American Society of Agricultural and Biological Engineers; 2011;54(3):1169–74.

97. Hameed BH, Lai LF, Chin LH. Production of biodiesel from palm oil (Elaeis guineensis) using heterogeneous catalyst: An optimized process. Fuel Process Technol. 2009;90(4):606–10.

98. Moser BR. Biodiesel production, properties, and feedstocks. Vitr Cell Dev Biol - Plant. 2009;45(3):229–66.

99. Islam RB., Islam R, Uddin MN., Ehsan M. Fuel properties and engine performance of biodiesel from waste cooking oil collected in Dhaka city. American Institute of Physics Conference Series. 2016.1754:50044.

100. Samavati M, Martin A, Santarelli M, Nemanova V. Synthetic diesel production as a form of renewable energy storage. Energies. 2018;11:1223.

101. Zeman P, Hönig V, Procházka P, Marik J. Dimethyl ether as a renewable fuel for diesel engines. Agron Res. 2017;15:2226–35.

102. Šebor G, Pospišil M, Žákovec J. Technical--economic analysis of suitable alternative fuels in transport, Ministry of Transport. 2006.

103. Azizi Z, Rezaeimanesh M, Tohidian T, Rahimpour MR. Dimethyl Ether: A Review of Technologies and Production Challenges. Chem Eng Process Process Intensif. 2014;82:150-72.

104. Chang J, Fu Y, Luo Z. Experimental study for dimethyl ether production from biomass gasification and simulation on dimethyl ether production. Biomass and bioenergy. 2012;39:67–72.

105. Research GV. Furfural Market Size, Share & Trends Analysis Report by Process, by Raw Material (Corncob, Sugarcane Bagasse), By Application (Furfuryl Alcohol, Solvent), By End Use (Pharmaceutical, Refineries), and Segment Forecasts,

2020-2027. 2020.

106. Sasaki M, Adschiri T, Arai K. Fractionation of sugarcane bagasse by hydrothermal treatment. Bioresour Technol. 2003;86(3):301–4.

107. Alonso DM, Wettstein SG, Mellmer MA, Gurbuz EI, Dumesic JA. Integrated conversion of hemicellulose and cellulose from lignocellulosic biomass. Energy Environ Sci. The Royal Society of Chemistry; 2013;6(1):76–80.

108. Brazdausks P, Vedernikovs N, Puke M, Kruma I. Effect of the Acid Hydrolysis Temperature on the Conversion of Birch Wood Hemicelluloses into Furfural. Key Eng Mater. 2014;604:245–8.

109. Wang T, Li K, Liu Q, Zhang Q, Qiu S, Long J, Chen L, Ma L, Zhang Q. Aviation fuel synthesis by catalytic conversion of biomass hydrolysate in aqueous phase. Appl Energy. 2014;136:775–80.

110. Luo Y, Li Z, Li X, Liu X, Fan J, Clark JH, Hu C. The production of furfural directly from hemicellulose in lignocellulosic biomass: A review. Catal Today. 2019;319:14–24.

111. Menegazzo F, Ghedini E, Signoretto M. 5-Hydroxymethylfurfural (HMF) Production from Real Biomasses. Molecules. 2018;23(9):2201.

112. Artz J, Palkovits R. Cellulose-based platform chemical: The path to application. Curr Opin Green Sustain Chem. 2018;14:14–8.

113. Ma Y, Wang L, Li H, Wang T, Zhang R. Selective Dehydration of Glucose into 5-Hydroxymethylfurfural by Ionic Liquid-ZrOCl2 in Isopropanol. Catalysts. 2018;8:467.

114. Gallo JMR, Alonso DM, Mellmer MA, Dumesic JA. Production and upgrading of 5-hydroxymethylfurfural using heterogeneous catalysts and biomass-derived solvents. Green Chem. 2013;15(1):85–90.

115. Salak Asghari F, Yoshida H. Acid-Catalyzed Production of 5-Hydroxymethyl Furfural from d-Fructose in Subcritical Water. Ind Eng Chem Res. 2006;45(7):2163–73.

116. Caes BR, Teixeira RE, Knapp KG, Raines RT. Biomass to Furanics: Renewable Routes to Chemicals and Fuels. ACS Sustain Chem Eng. 2015;3(11):2591–605.

117. Akmaz S, Esen M, Sezgin E, Koc SN. Efficient manganese decorated cobalt based catalysts for hydrogenation of 5-hydroxymethylfurfural (HMF) to 2,5-

dimethylfuran (DMF) biofuel. Can J Chem Eng. 2020;98(1):138–46.

118. Hackl R, Harvey S. Opportunities for Process Integrated Biorefinery Concepts in the Chemical Cluster in Stenungsund. 2010.

119. Chemmangattuvalappil NG, Ng DKS. A systematic methodology for optimal product design in an integrated biorefinery. In: Kraslawski A, Turunen IBT-CACE, editors. 23 European Symposium on Computer Aided Process Engineering. 201332:91–6.

120. Zhang W-H, Kaur I, Zhang W, Shen J, Ni Y. Recovery of manool from evaporator condensate by induced air flotation in a kraft pulp mill based integrated biorefinery. Sep Purif Technol. 2017;188:508–11.

121. Ajao O, Restrepo A, El Mehdi N, Boumghar Y, Benali M. Yellow birch transformation into butanol and organic acids in an integrated biorefinery platform. NWBC. 2015;255–9.

122. Guigou MD, Cebreiros F, Cabrera MN, Ferrari MD, Lareo C. Bioethanol production from Eucalyptus grandis hemicellulose recovered before kraft pulping using an integrated biorefinery concept. Biomass Convers Biorefinery. 2017;7(2):191–7.

123. Mendes CVT, Carvalho MGVS, Baptista CMSG, Rocha JMS, Soares BIG, Sousa GDA. Valorisation of hardwood hemicelluloses in the kraft pulping process by using an integrated biorefinery concept. Food Bioprod Process. 2009;87(3):197–207.

124. Li J, Zhang C, Hu H, Chai X-S. Effect of shortening kraft pulping integrated with extended oxygen delignification on biorefinery process performance of eucalyptus. Bioresour Technol. 2016;202:119–24.

125. Chen X, Zhang K, Xiao L-P, Sun R-C, Song G. Total utilization of lignin and carbohydrates in Eucalyptus grandis: an integrated biorefinery strategy towards phenolics, levulinic acid, and furfural. Biotechnol Biofuels. 2020;13(1):2.

126. Yoon S-H, van Heiningen A. Green liquor extraction of hemicelluloses from southern pine in an Integrated Forest Biorefinery. J Ind Eng Chem. 2010;16(1):74–80.

127. Toledano A, Serrano L, Labidi J. Enhancement of Lignin Production from Olive Tree Pruning Integrated in a Green Biorefinery. Ind Eng Chem Res.

2011;50(11):6573–9.

128. Romaní A, Ruiz HA, Teixeira JA, Domingues L. Valorization of Eucalyptus wood by glycerol-organosolv pretreatment within the biorefinery concept: An integrated and intensified approach. Renew Energy. 2016;95:1–9.

129. Hundt M, Engel N, Schnitzlein K, Schnitzlein MG. The AlkaPolP process: Fractionation of various lignocelluloses and continuous pulping within an integrated biorefinery concept. Chem Eng Res Des. 2016;107:13–23.

130. Tian D, Hu J, Chandra RP, Saddler JN, Lu C. Valorizing Recalcitrant Cellulolytic Enzyme Lignin via Lignin Nanoparticles Fabrication in an Integrated Biorefinery. ACS Sustain Chem Eng. 2017;5(3):2702–10.

131. Amezcua-Allieri M, Aburto J. Conversion of Lignin to Heat and Power, Chemicals or Fuels into the Transition Energy Strategy. Lignin-Trends and Applications. 2018.

132. Välimäki E, Niemi P, Haaga K. A Case Study on the Effects of Lignin Recovery on Recovery Boiler Operation. TAPPI/PAPTAC International Chemical Recovery Conference. 2010;148-56.

133. Davis KM, Rover M, Brown RC, Bai X, Wen Z, Jarboe LR. Recovery and utilization of lignin monomers as part of the biorefinery approach. Energies. 2016;9(10):808.

134. Cebreiros F, Clavijo L, Boix E, Ferrari MD, Lareo C. Integrated valorization of eucalyptus sawdust within a biorefinery approach by autohydrolysis and organosolv pretreatments. Renew Energy. 2020;149:115–27.

135. Ghosh D, Dasgupta D, Agrawal D, Kaul S, Adhikari DK, Kurmi AK, Arya PK, Bangwal D, Negi MS. Fuels and Chemicals from Lignocellulosic Biomass: An Integrated Biorefinery Approach. Energy & Fuels. 2015;29(5):3149–57.

136. Schmidt LM, Mthembu LD, Reddy P, Deenadayalu N, Kaltschmitt M, Smirnova I. Levulinic acid production integrated into a sugarcane bagasse based biorefinery using thermal-enzymatic pretreatment. Ind Crops Prod. 2017;99:172–8.

137. Sartori GP, Vasconcelos MH, Santos JC, Milagres AMF, Ferraz A. An innovative concept for industrial sugarcane processing enhances polysaccharide utilization in first- and second-generation integrated biorefineries. Ind Crops Prod. 2019;141:111801.

138. Santos DT, Albarelli JQ, Rostagno MA, Ensinas A V, Maréchal F, Meireles MAA.

New proposal for production of bioactive compounds by supercritical technology integrated to a sugarcane biorefinery. Clean Technol Environ Policy. 2014;16(7):1455–68.

139. Martínez-Guido SI, Betzabe González-Campos J, Ponce-Ortega JM, Nápoles-Rivera F, El-Halwagi MM. Optimal reconfiguration of a sugar cane industry to yield an integrated biorefinery. Clean Technol Environ Policy. 2016;18(2):553–62.

140. Cárdenas-Fernández M, Bawn M, Hamley-Bennett C, Bharat PK V, Subrizi F, Suhaili N, Ward DP, Bourdin S, Dalby PA, Hailes HC, Hewitson P. An integrated biorefinery concept for conversion of sugar beet pulp into value-added chemicals and pharmaceutical intermediates. Faraday Discuss. 2017;202:415–31.

141. Suhaili N, Cárdenas-Fernández M, Ward JM, Lye GJ. Potential of sugar beet vinasse as a feedstock for biocatalyst production within an integrated biorefinery context. J Chem Technol Biotechnol. 2019;94(3):739–51.

142. Du C, Rios-Solis L, Ward J, Dalby P, Lye G. Evaluation of CV2025 ω-transaminase for the bioconversion of lignin breakdown products into value-added chemicals: Synthesis of vanillylamine from vanillin. Biocatal Biotransformation. 2014;32(5-6):302-13.

143. Han J, Cao R, Zhou X, Xu Y. An integrated biorefinery process for adding values to corncob in co-production of xylooligosaccharides and glucose starting from pretreatment with gluconic acid. Bioresour Technol. 2020;307:123200.

144. Zhou H, Zhan W, Wang L, Guo L, Liu Y. Making Sustainable Biofuels and Sunscreen from Corncobs To Introduce Students to Integrated Biorefinery Concepts and Techniques. J Chem Educ. 2018;95(8):1376–80.

145. Gandarias I, García-Fernández S, Obregón I, Agirrezabal-Telleria I, Arias PL. Production of 2-methylfuran from biomass through an integrated biorefinery approach. Fuel Process Technol. 2018;178:336–43.

146. Li Y, Cao X-F, Sun S-N, Yuan T-Q, Wen J-L, Wang X-L, Xiao L-P, Sun R-C. An integrated biorefinery process to comprehensively utilize corn stalk in a MIBK/water/Al(NO3(3.9H2) biphasic system: Chemical and morphological changes. Ind Crops Prod. 2020;147:112173.

147. Yang S, Zhang Y, Yue W, Wang W, Wang Y-Y, Yuan T-Q, Sun R-C. Valorization of lignin and cellulose in acid-steam-exploded corn stover by a moderate alkaline ethanol post-treatment based on an integrated biorefinery concept. Biotechnol

Biofuels. 2016;9(1):238.

148. Liu X, Liu H, Yan P, Mao L, Xu Z, Zhang ZC. Mechanocatalytic Synergy for Expedited Cellulosic Ethanol Production Compatible with Integrated Biorefinery. ACS Sustain Chem Eng. 2020;8(6):2399–408.

149. Xia F, Liu H, Lu J, Lv Y, Zhai S, An Q, Cheng Y, Wang H. An integrated biorefinery process to produce butanol and pulp from corn straw. Ind Crops Prod. 2019;140:111648.

150. Budarin VL, Shuttleworth PS, Dodson JR, Hunt AJ, Lanigan B, Marriott R, Milkowski KJ. Use of green chemical technologies in an integrated biorefinery. Energy Environ Sci. 2011;4(2):471–9.

151. Morais ARC, Mata AC, Bogel-Lukasik R. Integrated conversion of agroindustrial residue with high pressure CO2 within the biorefinery concept. Green Chem. 2014;16(9):4312–22.

152. Nuruddin M, Chowdhury A, Haque S, Rahman M, Farhad SFU, Jahan M, Quaiyyum A. Extraction and Characterization of Cellulose Microfibrils from Agricultural Wastes in an Integrated Biorefinery Initiative. Cellul Chem Technol. 2011;45:347–54.

153. Jahan MS, Sultana N, Rahman M, Quaiyyum A. An integrated biorefinery initiative in producing dissolving pulp from agricultural wastes. Biomass Convers Biorefinery. 2013;3(3):179–85.

154. Ali AAM, Othman MR, Shirai Y, Hassan MA. Sustainable and integrated palm oil biorefinery concept with value-addition of biomass and zero emission system. J Clean Prod. 2015;91:96–9.

155. Yusof SJHM, Roslan AM, Ibrahim KN, Abdullah SSS, Zakaria MR, Hassan M, Shirai Y. Environmental performance of bioethanol production from oil palm frond petiole sugars in an integrated palm biomass biorefinery. IOP Conf Ser Mater Sci Eng. 2018;368:12004.

156. Burhani D, Triwahyuni E. An integrated biorefinery process of oil palm empty fruit bunch for bioethanol and flame retardant. AIP Conference Proceedings. 2019;2175(1):20034.

157. Schievano A, Adani F, Buessing L, Botto A, Casoliba EN, Rossoni M, Goldfarb JL. An integrated biorefinery concept for olive mill waste management:

supercritical CO2 extraction and energy recovery. Green Chem. 2015;17(5):2874–87.

158. Goldfarb JL, Buessing L, Gunn E, Lever M, Billias A, Casoliba E, Schievano A, Adani F. Novel Integrated Biorefinery for Olive Mill Waste Management: Utilization of Secondary Waste for Water Treatment. ACS Sustain Chem Eng. 2017;5(1):876–84.

159. Zhu Q, Shao R, Dong R, Yun Z. An integrated approach for obtaining biodiesel, sterols, gossypol, and raffinose from cottonseed on a biorefinery concept. Energy. 2014;70:149–58.

160. Dávila I, Gordobil O, Labidi J, Gullón P. Assessment of suitability of vine shoots for hemicellulosic oligosaccharides production through aqueous processing. Bioresour Technol. 2016;211:636–44.

161. Kachrimanidou V, Kopsahelis N, Alexandri M, Strati A, Gardeli C, Papanikolaou S, Komaitis M, Kookos IK, Koutinas AA. Integrated sunflower-based biorefinery for the production of antioxidants, protein isolate and poly(3-hydroxybutyrate). Ind Crops Prod. 2015;71:106–13.

162. Sekhon JK, Maurer D, Wang T, Jung S, Rosentrater KA. Ethanol production by soy fiber treatment and simultaneous saccharification and co-fermentation in an integrated corn-soy biorefinery. Fermentation. 2018;4:35.

163. Gullón P, Gullón B, Muñiz-Mouro A, Lú-Chau TA, Eibes G. Valorization of horse chestnut burs to produce simultaneously valuable compounds under a green integrated biorefinery approach. Sci Total Environ. 2020;730:139143.

164. Kachrimanidou V, Kopsahelis N, Vlysidis A, Papanikolaou S, Kookos IK, Monje Martínez B, Rondan MCE, Koutinas AA. Downstream separation of poly(hydroxyalkanoates) using crude enzyme consortia produced via solid state fermentation integrated in a biorefinery concept. Food Bioprod Process. 2016;100:323–34.

165. Dávila I, Gullón P, Andrés MA, Labidi J. Coproduction of lignin and glucose from vine shoots by eco-friendly strategies: Toward the development of an integrated biorefinery. Bioresour Technol. 2017;244:328–37.

166. Lupitskyy R, Staff C, Satyavolu J. Towards integrated biorefinery from dried distillers grains: Evaluation of feed application for Co-products. Biomass and Bioenergy. 2015;72:251–5.

167. Fonseca DA, Lupitskyy R, Timmons D, Gupta M, Satyavolu J. Towards integrated biorefinery from dried distillers grains: Selective extraction of pentoses using dilute acid hydrolysis. Biomass and Bioenergy. 2014;71:178–86.

168. Naranjo JM, Cardona CA, Higuita JC. Use of residual banana for polyhydroxybutyrate (PHB) production: Case of study in an integrated biorefinery. Waste Manag. 2014;34(12):2634–40.

169. Talekar S, Patti AF, Vijayraghavan R, Arora A. An integrated green biorefinery approach towards simultaneous recovery of pectin and polyphenols coupled with bioethanol production from waste pomegranate peels. Bioresour Technol. 2018;266:322–34.

170. Dlangamandla N, Ntwampe SK, Angadam JO, Itoba-Tombo EF, Chidi BS, Mekuto L. Integrated Hydrolysis of Mixed Agro-Waste for a Second Generation Biorefinery Using Nepenthes mirabilis Pod Digestive Fluids. Processes. 2019;7(2):64.

171. Tsouko E, Maina S, Ladakis D, Kookos IK, Koutinas A. Integrated biorefinery development for the extraction of value-added components and bacterial cellulose production from orange peel waste streams. Renew Energy. 2020;160:944–54.

172. Ebikade E, Athaley A, Fisher B, Yang K, Wu C, Ierapetritou MG, Vlachos DG. The Future is Garbage: Repurposing of Food Waste to an Integrated Biorefinery. ACS Sustain Chem Eng. 2020;8(22):8124–36.

173. Zhang C, Sanders JPM, Xiao TT, Bruins ME. How Does Alkali Aid Protein Extraction in Green Tea Leaf Residue: A Basis for Integrated Biorefinery of Leaves. PLoS One. 2015;10(7):1–14.

174. Ichsan, Hadiyanto H, Hendroko R. Integrated Biogas-microalgae from Waste Waters as the Potential Biorefinery Sources in Indonesia. Energy Procedia. 2014;47:143–8.

175. Chavan R, Mutnuri S. Tertiary treatment of domestic wastewater by Spirulina platensis integrated with microalgal biorefinery. Biofuels. 2019;10(1):33–44.

176. Alexandri M, Papapostolou H, Komaitis M, Stragier L, Verstraete W, Danezis GP, Georgiou CA, Papanikolaou S, Koutinas AA. Evaluation of an integrated biorefinery based on fractionation of spent sulphite liquor for the production of an antioxidant-rich extract, lignosulphonates and succinic acid. Bioresour Technol. 2016;214:504–13.

177. Zambare VP, Christopher LP. Integrated biorefinery approach to utilization of pulp and paper mill sludge for value-added products. J Clean Prod. 2020;274:122791.

178. Rosenberg JN, Mathias A, Korth K, Betenbaugh MJ, Oyler GA. Microalgal biomass production and carbon dioxide sequestration from an integrated ethanol biorefinery in Iowa: A technical appraisal and economic feasibility evaluation. Biomass and Bioenergy. 2011;35(9):3865–76.

179. Girotto F, Pivato A, Cossu R, Nkeng GE, Lavagnolo MC. The broad spectrum of possibilities for spent coffee grounds valorisation. J Mater Cycles Waste Manag. 2017;20(1):695-701.

180. United States Department of Agriculture. Coffee: World markets and trade. 2020.

181. Bals B, Teachworth L, Dale B, Balan V. Extraction of Proteins from Switchgrass Using Aqueous Ammonia within an Integrated Biorefinery. Appl Biochem Biotechnol. 2007;143(2):187–98.

182. Sun S-L, Wen J-L, Ma M-G, Song X-L, Sun R-C. Integrated biorefinery based on hydrothermal and alkaline treatments: Investigation of sorghum hemicelluloses. Carbohydr Polym. 2014;111:663–9.

183. Sun S-L, Wen J-L, Ma M-G, Sun R-C. Structural Elucidation of Sorghum Lignins from an Integrated Biorefinery Process Based on Hydrothermal and Alkaline Treatments. J Agric Food Chem. 2014;62(32):8120–8.

184. Wu G, Zhang Y, Yuan B, Liu C, Yu G, Xie C, Li B, Peng H. Production of dissolving pulp from Eulaliopsis binata with the concept of integrated biorefinery. Cellulose. 2019;26(3):2087–97.

185. Popy RS, Ni Y, Salam A, Jahan MS. Mild potassium hydroxide-based alkaline integrated biorefinery process of Kash (Saccharum spontaneum). Ind Crops Prod. 2020;154:112738.

186. Kuglarz M, Alvarado-Morales M, Karakashev D, Angelidaki I. Integrated production of cellulosic bioethanol and succinic acid from industrial hemp in a biorefinery concept. Bioresour Technol. 2016;200:639–47.

187. Villegas Calvo M, Colombo B, Corno L, Eisele G, Cosentino C, Papa G, Scaglia B, Pilu R, Simmons B, Adani F. Bioconversion of Giant Cane for Integrated Production of Biohydrogen, Carboxylic Acids, and Polyhydroxyalkanoates (PHAs) in a Multistage Biorefinery Approach. ACS Sustain Chem Eng. 2018;6(11):15361–

73.

188. Schwarz D, Dörrstein J, Kugler S, Schieder D, Zollfrank C, Sieber V. Integrated biorefinery concept for grass silage using a combination of adapted pulping methods for advanced saccharification and extraction of lignin. Bioresour Technol. 2016;216:462–70.

189. Schwarz D, Schoenenwald AKJ, Dörrstein J, Sterba J, Kahoun D, Fojtíková P, Vilimek J, Schieder D, Zollfrank C, Sieber V. Biosynthesis of poly-3-hydroxybutyrate from grass silage by a two-stage fermentation process based on an integrated biorefinery concept. Bioresour Technol. 2018;269:237–45.

190. Kurniawan A, Effendi C, Ong LK, Ju Y-H, Lin CX, Ismadji S. Novel, Integrated Biorefinery Approach of Ceiba pentandra (Kapok) Seed and Its Secondary Waste. ACS Sustain Chem Eng. 2013;1(5):473–80.

191. Chen J, Bian X, Rapp G, Lang J, Montoya A, Trethowan R, Bouyssiere B, Portha JF, Jaubert JN, Pratt P, Coniglio L. From ethyl biodiesel to biolubricants: Options for an Indian mustard integrated biorefinery toward a green and circular economy. Ind Crops Prod. 2019;137:597–614.

192. Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: A review. Renew Sustain Energy Rev. 2010;14(1):217–32.

193. Spolaore P, Joannis-Cassan C, Duran E, Isambert A. Commercial applications of microalgae. J Biosci Bioeng. 2006;101(2):87–96.

194. Borowitzka MA. Commercial production of microalgae: ponds, tanks, and fermenters. Progress in industrial microbiology. 1999;35:313–21.

195. Sheehan J, Dunahay T, Benemann J, Roessler P. Look Back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from Algae; Close-Out Report. 1998.

196. Moheimani NR. The culture of coccolithophorid algae for carbon dioxide bioremediation. Doctoral dissertation. 2005.

197. Dong T, Van Wychen S, Nagle N, Pienkos PT, Laurens LML. Impact of biochemical composition on susceptibility of algal biomass to acid-catalyzed pretreatment for sugar and lipid recovery. Algal Res. 2016;18:69–77.

198. Shuba ES, Kifle D. Microalgae to biofuels: 'Promising' alternative and renewable energy, review. Renew Sustain Energy Rev. 2018;81:743–55.

199. Mercer P, Armenta RE. Developments in oil extraction from microalgae. Eur J Lipid Sci Technol. 2011;113(5):539–47.

200. Venkata Subhash G, Venkata Mohan S. Deoiled algal cake as feedstock for dark fermentative biohydrogen production: An integrated biorefinery approach. Int J Hydrogen Energy. 2014;39(18):9573–9.

201. Patnaik R, Mallick N. Utilization of Scenedesmus obliquus biomass as feedstock for biodiesel and other industrially important co-products: An integrated paradigm for microalgal biorefinery. Algal Res. 2015;12:328–36.

202. Esquivel Hernández D, Pennacchio A, Parra R, Faraco V. Integrated biorefinery from Arthrospira platensis biomass as feedstock for bioethanol and lactic acid production. bioRxiv. 2019:715672.

203. Dong T, Knoshaug EP, Davis R, Laurens LML, Van Wychen S, Pienkos PT, Nagle N. Combined algal processing: A novel integrated biorefinery process to produce algal biofuels and bioproducts. Algal Res. 2016;19:316–23.

204. Zhang W, Wang F, Gao B, Huang L, Zhang C. An integrated biorefinery process: Stepwise extraction of fucoxanthin, eicosapentaenoic acid and chrysolaminarin from the same Phaeodactylum tricornutum biomass. Algal Res. 2018;32:193–200.

205. Mishra S, Mohanty K. Co-HTL of domestic sewage sludge and wastewater treatment derived microalgal biomass – An integrated biorefinery approach for sustainable biocrude production. Energy Convers Manag. 2020;204:112312.

206. Ashokkumar V, Chen W-H, Kamyab H, Kumar G, Al-Muhtaseb AH, Ngamcharussrivichai C. Cultivation of microalgae Chlorella sp. in municipal sewage for biofuel production and utilization of biochar derived from residue for the conversion of hematite iron ore (Fe2O3) to iron (Fe) – Integrated algal biorefinery. Energy. 2019;189:116128.

207. Chaudhury NR, Sanghvi D, Jain B. Macroalgae Species as Zonal Indicators of Coral Reef: A Case Study from Bet Shankhodhar Reef, India. Wetlands Management-Assessing Risk and Sustainable Solutions. 2018.

208. Nations F and AO of the U. The state of World Fisheries and Aquaculture - Sustainability in action. 2020.

209. Beacham TA, Cole IS, DeDross LS, Raikova S, Chuck CJ, Macdonald J, Herrera L, Ali T, Airs RL, Landels A, Allen MJ. Analysis of seaweeds from South West

England as a biorefinery feedstock. Appl Sci. 2019;9:4456.

210. Parthiban C, Saranya C, Girija K, Hemalatha A, Suresh M, Anantharaman P. Biochemical composition of some selected seaweeds from Tuticorin coast. Adv Appl Sci Res. 2013;4(3):362–6.

211. Rohani-Ghadikolaei K, Abdulalian E, Ng W-K. Evaluation of the proximate, fatty acid and mineral composition of representative green, brown and red seaweeds from the Persian Gulf of Iran as potential food and feed resources. J Food Sci Technol. 2012;49(6):774–80.

212. Manivannan K, Thirumaran G, Devi GK, Hemalatha A, Anantharaman P. Biochemical composition of seaweeds from Mandapam coastal regions along Southeast Coast of India. Am J Bot. 2008;1(2):32–7.

213. Prabhu MS, Israel A, Palatnik RR, Zilberman D, Golberg A. Integrated biorefinery process for sustainable fractionation of Ulva ohnoi (Chlorophyta): process optimization and revenue analysis. J Appl Phycol. 2020;1-12.

214. Dohaei M, Karimi K, Rahimmalek M, Satari B. Integrated biorefinery of aquatic fern Azolla filiculoides for enhanced extraction of phenolics, protein, and lipid and methane production from the residues. J Clean Prod. 2020;276:123175.

215. Bruijnincx PCA, Weckhuysen BM. Lignin up for break-down. Nat Chem. 2014;6(12):1035–6.

216. Sun Z, Fridrich B, de Santi A, Elangovan S, Barta K. Bright Side of Lignin Depolymerization: Toward New Platform Chemicals. Chem Rev. 2018;118(2):614–78.

217. Xu C, Arancon RAD, Labidi J, Luque R. Lignin depolymerisation strategies: towards valuable chemicals and fuels. Chem Soc Rev. 2014;43(22):7485–500.

Chapter 2

The optimized production of 5-HMF and related bulk chemicals from spent coffee grounds

2.1 Context

Tackling climate change requires sustained and immediate action from national governments in reducing emissions. This includes changes not only in the transport and industrial sectors but also in waste management. Large amounts of waste are produced worldwide, leading directly to elevated greenhouse gas emissions. As such, waste is the fourth largest source of greenhouse gas emissions, after the transport, agricultural and industrial sectors.

One plausible solution to address this problem is the reuse of biogenic wastes, producing an array of products that are typically sourced from petrochemicals. One such waste are spent coffee grounds (SCG), with a range of work demonstrating the suitability of this feedstock for the production of biofuels. This is in part due to the diverse composition of SCG, which is rich in important biomolecules such as carbohydrates, lipids, lignin and protein, as well as being simple to process as the material has already been heavily broken down.

To this end, in the following chapter, an integrated biorefinery using SCG as a feedstock is presented, producing 5-hydroxymethylfurfural (HMF) alongside other value products. When published, this study was the first time SCG were processed in an integrated biorefinery approach, and is the first reported production of HMF from SCG. HMF is one of the most valuable building blocks in the nascent biorefinery industry as it has the potential to be converted into dimethylfuran, a potential greener replacement of gasoline, and 2,5-furandicarboxylic acid, the monomer used in the production of polyethylene furanoate (PEF), a replacement of polyethylene terephthalate (PET) in the plastic industry.

This chapter is submitted in an alternative format in line with Appendix 6A of the “Specifications for Higher Degree Theses and Portfolios” as required by the University of Bath.

All the experimental work in this chapter was undertaken by the author with the exception of the following:

- 2D Nuclear Magnetic Resonance (NMR) was undertaken by Dr. Tim Woodman, a co-author in this paper.
- Fermentation experiments were conducted in collaboration with Paraj Brahmbhatt, a co-author in this paper.
- Solid state NMR was performed by the analytical department personnel at Durham University, UK.

- ICP-OES was carried out by the analytical department personnel at Medac Pharma, UK.

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2.2 Applied Sciences paper

The optimized production of 5-HMF and related bulk chemicals from spent coffee grounds

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2.2.1 Key words

Spent coffee grounds

Biomass

Fractionation

Cellulose

Hemicellulose

Lignin

5-hydroxymethylfurfural

2.2.2 Abstract

The increasing consumption of coffee worldwide has led to higher amounts of spent coffee grounds (SCG) being produced which are generally disposed of in landfill or used as compost. However, the wide range of molecules present in SCG such as saccharides, lignin, lipids and proteins give this biomass source a large chemical functionality. In this work, SCG were fractionated to separate the components into three separate portions for further valorization; these were a hemicellulose-enriched fractions (HEF), lignin-enriched fraction (LEF) and cellulose-enriched fraction (CEF). HEF was effectively used in the growth of the oleaginous yeast strain *Metschnikowia pulcherrima*, additionally, the C_6 sugars present in this fraction suggests that it can be used in the production of 5-hydroxymethylfurfural (HMF). The LEF had a considerable high heating value (HHV) and would be suitable as a biofuel component for combustion. CEF was efficiently used in the production of HMF as 0.35 g of this product were obtained from 10 g of SCG. Such results demonstrate that SCG can be effectively used in the production of HMF within a biorefinery concept.

2.2.3 Introduction

Coffee is one of the most consumed beverages in the world and as such is becoming a daily commodity in most people's lives (1). Such high consumption of coffee generates increasing amounts of spent coffee grounds (SCG). The United States Department of Agriculture estimate the yearly consumption to be 9.8 million tonnes of coffee beans worldwide (2), which has been increasing steadily since 2011 (2-4). This demand for coffee demonstrates the lack of seasonality in the supply of coffee beans and the increasing availability of SCG to be industrially used and converted to fuels and high value chemicals.

SCG were initially considered a waste, being landfilled or disposed in the sea (5,6). However, the compositional analysis reveals the presence of carbohydrates (42-55 % w/w), lignin (0-25 % w/w), lipids (2-24 % w/w), protein (10-18 % w/w), caffeine (0-0.4 % w/w), chlorogenic acids (1-3 % w/w) and ash (1-2 % w/w) (7-12). This composition lends itself to the production of fuels and higher value chemicals, and as such is a highly suitable feedstock to be used in the biorefinery industry.

Initially, as the calorific value of SCG were considered to be similar to agro-chemical residues SCG were used directly for heat generation in industrial boilers (13). However, due to the increased heteroatom content, emissions from direct combustion are particularly hazardous. More suitably SCG have been pyrolyzed, yielding between 55% and 85% bio-oil, depending on the reaction conditions and moisture content (14). The pyrolysis process is particularly attractive and as such higher value compounds such as diterpenes can be extracted from the bio-oil. To this end, Peshev et al. added a downstream nanofiltration process that used the permeate and retentate in the production of further products (15,16).

The pyrolysis yields are so high as SCG contain up to 15% triglyceride oil. Alternative work has therefore focused on the extraction and transesterification of these lipids into biodiesel (14, 17-19). However, the low triglyceride oil content in SCG requires large amounts of SCG to produce considerable amounts of biodiesel, and as such cannot be produced as economically as from other glyceride sources.

Due to the high carbohydrate content, SCG have also been used in the production of ethanol through fermentation. Mussatto *et al.* used an acid pretreatment of the SCG, followed by enzymatic step to release the monosaccharides. The authors compared three different yeast strains on both SCG and coffee silverskin. The SCG used led to much higher ethanol productions (11.7 g/L) compared to the coffee silverskin (producing less than 1 g/L) (20). Rocha et al, developed a biorefinery approach through fermentation

of the SCG after the extraction of oil. This new design led to higher ethanol concentrations with a final titer of 19 g/L, while the extracted oil was used in the production of biodiesel.

However, with the unique diversity of molecules present in SCG there is also a large potential to produce higher value chemicals alongside fuels. Burniol-Figols et al. developed a biorefinery design to produce chlorogenic acid and bioethanol (21). The process included the extraction of phenolics to be converted into chlorogenic acid where the extraction residues were submitted to an acid hydrolysis, to depolymerize the sugars into monosaccharides, followed by fermentation to ethanol. Karmee evaluated a wide range of processes to come up with SCG-based biorefinery design (10). The evaluated processes included: different oil extraction processes, the production of biodiesel through base, acid, lipase catalysis or in-situ transesterification of the extracted oils, the catalytical upgrading of the oils to renewable diesel, production of bioethanol through hydrolysis and fermentation of the carbohydrates. The author also reported additional products such as carotenoids, antioxidants and polymers, such as polyols, polylactic acid and polyhydroxyalkanoates.

Caetano *et al.* presented a design composed of two initial extractions, one aqueous and one lipophilic, followed by the fermentation of the remaining solid waste (22). The first extraction was intended to remove high value extracts for upgrading to pharmaceuticals or for the cosmetic industry, while the product of the second extraction were triglycerides, used in the production of biodiesel. Using a similar design, Mata *et al.* replaced the fermentation process by pyrolysis and torrefaction to produce biochar and bio-oil (23).

While the C6 sugars can be fermented into a range of products, another application is in the acid catalyzed dehydration to produce 5-hyroxymethylfurfural (HMF) (24). HMF can be used in a wide range of applications such as polymers and biofuels. Two of the most common applications include dimethylfuran (DMF) and 2,5-furandicarboxylic acid (FDCA). While DMF is known for being a potential biofuel with comparable energy density to gasoline, FDCA is a building block used in the production of polyesters, one of which, PEF, is a potential replacement for PET (25,26). To date, however, there are no reports of HMF being produced from SCG.

In this work, a conceptual HMF biorefinery was investigated with the SCG being fractionated to separate the biomass into three different fractions: a cellulose-enriched fraction (CEF), hemicellulose-enriched fraction (HEF) and lignin-enriched fraction (LEF). This process has been developed for other lignocellulosic biomass, though has again not been demonstrated on SCG, and uses sulfuric acid as a catalyst and a ternary

mixture of methyl isobutyl ketone (MIBK), ethanol and water to separate the biomass into the various fractions (27). More recent work done by Katahira *et al.*, demonstrated that the replacement of ethanol in the ternary system with acetone leads to a more effective fractionation process (28). In this investigation, these solvent systems were then examined for the suitability to produce multiple product streams from SCG, with a focus on the production of HMF.

2.2.4 Materials and Methods

2.2.4.1 Materials

The SCG were obtained from Bio-bean Ltd. They were stored at 4 °C. SCG are commonly composed by cellulose (10-13 % w/w), hemicellulose (32-42 % w/w), lignin (0-25 % w/w), protein (10-18 % w/w), lipids (2-24 % w/w), ash (1-2 % w/w), caffeine (0-0.4 % w/w) and chlorogenic acids (1-3 % w/w) (8-12). Pistachio hull was supplied by the Wonderful Company (Los Angeles, CA, USA), the compositional analysis is given in the Appendix A (Table 2-9). All the chemicals used in the were purchased from Sigma Aldrich (Gillingham, UK), and used without further purification.

2.2.4.2 Clean fractionation

The fractionation process was carried out in a 300 mL Parr reactor (Parr Company Moline, IL, USA, 4560 mini reactors). In this process, 10 g of biomass were fractionated in three different solvent systems: (1) methyl isobutyl ketone (MIBK), ethanol and water (16/34/50 g/g/g; here forth this solvent system is denominated MEW); (2) MIBK, acetone and water (11/44/44 g/g/g, denominated MAW); (3) MIBK and water (16/84 g/g; MW). In all these systems, sulfuric acid was used as a catalyst in a concentration of 0.1 M. After loading both the biomass and solvent system, agitation was started, temperature increased to 140 °C and the reaction was carried out for 1 hour. After the reaction, the reactor was cooled down to 30 °C. The obtained suspension was filtered and washed, initially with 200 mL of the same solvent system used in the fractionation, and secondly, with 650 mL of water to remove any soluble component present amidst the solids. The solid residue after filtration was denominated the cellulose-enriched fraction (CEF) and was dried at room temperature for 24 hours. Then, 50 mL of MIBK were added to the filtrate in a separatory funnel. The solution was mixed and left to rest until two distinct phases were observed (an aqueous and an organic phase). Once the phases were separated, 50 mL of MIBK were added to the aqueous phase for a second extraction. The solution was mixed, left to rest and the two distinct phases were separated. The obtained aqueous phase was denominated the hemicellulose-enriched fraction (HEF). Both organic phases (obtained from first and second extraction processes) were combined and the solvent removed under vacuum. The dried solids obtained from this

process were further dried in an oven at 40 °C for 4 days, this was designated the lignin-enriched fraction (LEF).

2.2.4.3 Fermentation

The pH of the HEF fraction was increased to 4. This solution was then used in the preparation of four diluted solutions with deionized water: 25% HEF concentrated, 50% HEF concentrated, 75% HEF concentrated and a fully concentrated solution (100%). A control media of 30 g/L of peptone from soybean meal enzymatic digest and 25 g/L of malt extract was also prepared and autoclaved. All these solutions (4 dilutions from HEF and the control) were then inoculated with *Metschnikowia pulcherrima* (National Culture of Yeast Collection, NCYC4331) to make solutions with 500,000 cells/mL and a total volume of 20 mL in Erlenmeyer flasks. The flasks were sealed and placed in incubators at a temperature of 25 °C and 230 rpm for 3 days.

2.2.4.4 2D NMR

Solution state nuclear magnetic resonance (NMR) spectra were acquired using a Bruker Avance III NMR spectrometer operating at 500.13 MHz for ^{1}H and 125.77 MHz for ^{13}C. Samples were investigated in DMSO-d6 at 27 °C unless specified and were referenced to the residual solvent signal at 2.50 ppm (^{1}H) and 39.52 ppm (^{13}C). Following this, ^{1}H-^{13}C correlation spectra were obtained using the “hsqcedetgpsp” pulse sequence, with td=256 and ns=128 and a relaxation delay of 1.5s. Spectra were acquired using Bruker Topspin 2.1, and processed using Bruker Topspin 3.1. Assignment of the individual components follows previous reports (28,29).

2.2.4.5 Cellulose hydrolysis

The enzymatic hydrolysis of cellulose was carried out in 50 mL Falcon tubes in an incubator at 50 °C. Then, 0.5 g of CEF were added to 5 mL of a 0.1 M buffer of sodium acetate and acetic acid with a pH of 4.8. Cellulase (cellulase from *Aspergillus niger* sourced from Sigma-Aldrich, UK) was used as enzyme – 137.04 FPU/mL. Antibiotics were also added to avoid contamination: 12 mg/L of tetracyclin and 15 mg/L of gentamicin. The reaction was carried out for 72 hours. At the end of the reaction, the solution was placed in a hot bath at 85 °C for enzyme deactivation, followed by centrifugation to separate the majority of the solids. High Performance Liquid Chromatography (HPLC) equipped with an Aminex HPX-87H HPLC column from Bio-Rad (heated up to 65 °C, with a flow rate of 0.6 mL/min) and a refractive index detector was used to determine the sugars content in solution.

2.2.4.6 Glucose isomerization

After hydrolysis, the buffer solution had to be neutralized to a pH of 7 as this is the ideal pH for glucose isomerase from *Streptomyces murinus* (Sigma-Aldrich, UK) – 15 FPU of enzyme were added to solution. The reaction was carried out for 24 hours at 60 °C. The same conditions (column and detector) used in the glucose analysis were used for fructose quantification. The glucose concentrations in the initial solution and the added volume of sodium acetate (used in neutralization) were taken into account in the fructose yield calculations.

2.2.4.7 Dehydration to HMF

The HMF production was carried out in a biphasic system composed of 1.5 g of sodium chloride saturated aqueous solution (5 wt% fructose, 5 mM of $AlCl_3$, 3.17 mM of HCl) and 3 g of γ-valerolactone (GVL) as the organic solvent. The reaction was performed in glass pressure tubes and heated up to 170 °C. After 20 minutes the pressure tubes were cooled down to room temperature. The two phases were separated using a separating funnel. The HMF present in GVL was extracted from this solvent by mixing 1 part of GVL with 1 part of water and 20 parts of cyclopentane in a first extraction. This led to an aqueous solution containing 90% of the produced HMF and 43% of GVL (30). By mixing the resulting aqueous phase with 20 parts of cyclopentane three more times, the percentage of GVL in the aqueous solution is reduced to 0.5% and the percentage of recovered HMF is 99% of the total produced. The same Aminex HPX-87H column was used in the HMF analysis, however a diode array detector was used at a wavelength of 280 nm. The 5 mM sulfuric acid aqueous solvent was set to a flow rate of 0.6 mL/min and the column heated up to 65 °C.

2.2.5 Results

The fractionation process was assessed in three different solvent systems. In the first MIBK, ethanol and water were used (MEW), which was compared to MIBK, acetone and water (MAW). Both the ethanol and acetone partitioned into the HEF and as these are inhibitory to microbes at these concentrations; a solvent system only composed of MIBK and water (MW) was also examined. For each one of these three solvent systems, two biomass sources were studied: SCG and pistachio hull, allowing the SCG to be compared to a lignocellulosic material. The initial mass balance for the three systems is given in Figure 2-1.

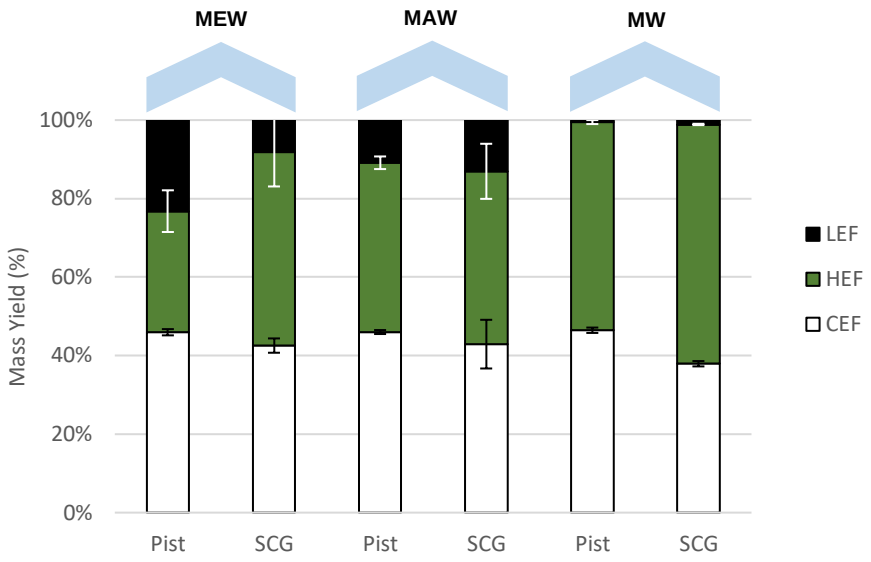
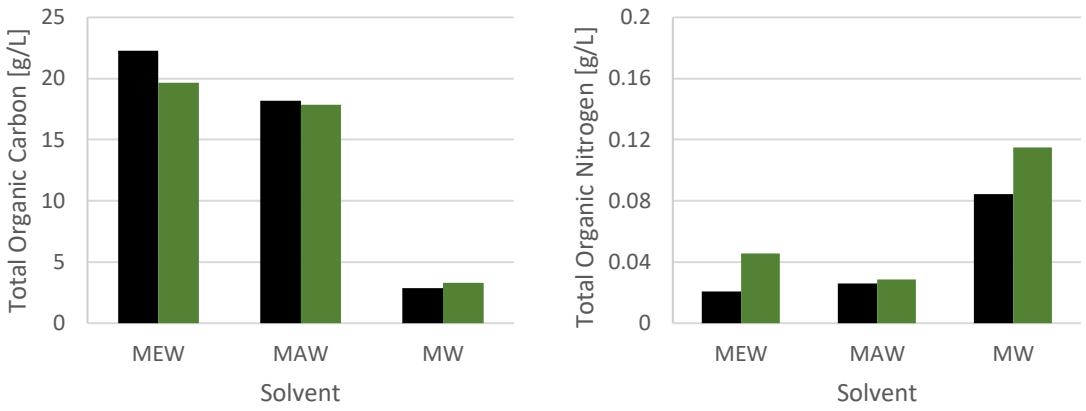


Figure 2-1 – Cellulose-enriched fraction (CEF), hemicellulose-enriched fraction (HEF) and lignin-enriched fraction (LEF) yields obtained in the six different types of fractionation with two feedstock (pistachio hull and spent coffee grounds, SCG) and three solvent systems (MEW – MIBK, methanol and water; MAW – MIBK, acetone and water; MW – MIBK and water).

2.2.5.1 Hemicellulose enriched fraction (HEF)

Characterization

To characterize the HEF, samples were submitted to different analysis methods, HPLC was used to evaluate what type of sugars and their quantities are present in this fraction. Total organic carbon (TOC) was performed to assess the amount of carbon present in the organic compounds (mainly carbohydrates) that can be used as a substrate to grow yeast/microalgae or to convert to furans. In addition to this, the amount of nitrogen in these fractions was obtained by a total nitrogen (TN) analysis (Figure 2-2). Finally, inductively coupled plasma optical emission spectrometry (ICP-OES) was used to quantify the metal composition of HEF samples to determine the suitability as a fermentation broth.



(a) (b)

Figure 2-2 – Total organic carbon (a) and total nitrogen (b) analysis performed on HEF samples obtained from the fractionation of pistachio hull (black) and SCG (green). The three solvent systems (MEW, MAW and MW) are compared in both analysis

All the processes release suitable levels of micronutrients into the broth for fermentation (Table 2-1). Using the MW solvent system for SCG led to increases in sulfur and reduced levels of Na, though all other elements were widely similar irrespective of the system used. Pistachio hulls had higher concentration of calcium and potassium regardless of the solvent system, in line with the elemental composition of the initial feedstock. HPLC analysis using a refractive index detector was used to quantify the concentration of the sugars present in this fraction (Table 2-2). Interestingly, while xylose (and possible arabinose which elutes at the same time) are present in the HEF produced from pistachio hulls, there were very few C_5 sugars produced from SCG. Instead the soluble HEF fraction contained high levels of galactose and mannose, in addition to glucose, maltose and cellobiose. This demonstrates that SCG would be entirely unsuitable for the production of furfural but rather that fermentation would be a suitable processing step for this fraction.

Table 2-1 – Summary of inductively coupled plasma optical emission spectrometry (ICP-OES) results performed to HEF samples

System	Ca (ppm)	K (ppm)	Mg (ppm)	Na (ppm)	P (ppm)	S (ppm)	Others (ppm)
Pist - MEW	46.1	123.3	42	503.8	6.9	123	6.0
SCG - MEW	13.2	56.1	6.2	355.5	7.4	137	10.3
Pist - MAW	37.9	130.9	1.9	480.2	6.3	105	7.1
SCG - MAW	21.7	37.0	8.2	10.7	9.9	134	5.8
Pist - MW	61.9	85.0	6.1	10.7	7.2	258	14.8
SCG - MW	16.1	24.3	7.4	3.9	7.4	225	5.7

Table 2-2 – Sugar content of HEF samples based on HPLC analysis

Solvent	Biomass	Cellobiose [g/L]	Maltose [g/L]	Glucose [g/L]	Xylose [g/L]	Galactose/Mannose [g/L]
MEW	Pist	4.65	0.11	0.0008	0.34	-
	SCG	-	1.15	0.0007	-	7.41
MAW	Pist	-	0.01	0.0007	0.37	-
	SCG	-	0.15	0.0007	-	8.21
MW	Pist	0.71	0.01	0.0015	4.12	-
	SCG	-	0.02	0.0010	-	18.74

To determine the suitability of HEF from SCG for fermentation, the substrate was used with *Metshnikowia pulcherrima*, an oleaginous yeast species which can ferment a large range of alternative sugars and has excellent inhibitor tolerance (31). The yeast could not grow on the MEW and MAW derived HEF fractions (data not shown) presumably due to the elevated levels of both ethanol and acetone in the broth. The number of cells per mL increased with time for all the samples (Figure 2-3 (a)). An increase in the concentration of the medium was directly proportional to an increase in the number of cells. At the end of the first day, the 25% concentrated medium achieved an increase of 1,204,375 cells per mL, the 50% concentrated medium increased by 2,530,000 in the number of cells per mL, the medium with 75% HEF registered an increase of 3,792,500 in the number of cells per mL while the fully concentrated medium increased by 7,665,625. This demonstrated that HEF was not toxic to the yeast and rather was limited by the carbon available. The fully concentrated medium therefore could potentially be used for the fermentation as long as no ethanol or acetone were used in this fractionation process, or were removed prior to use.

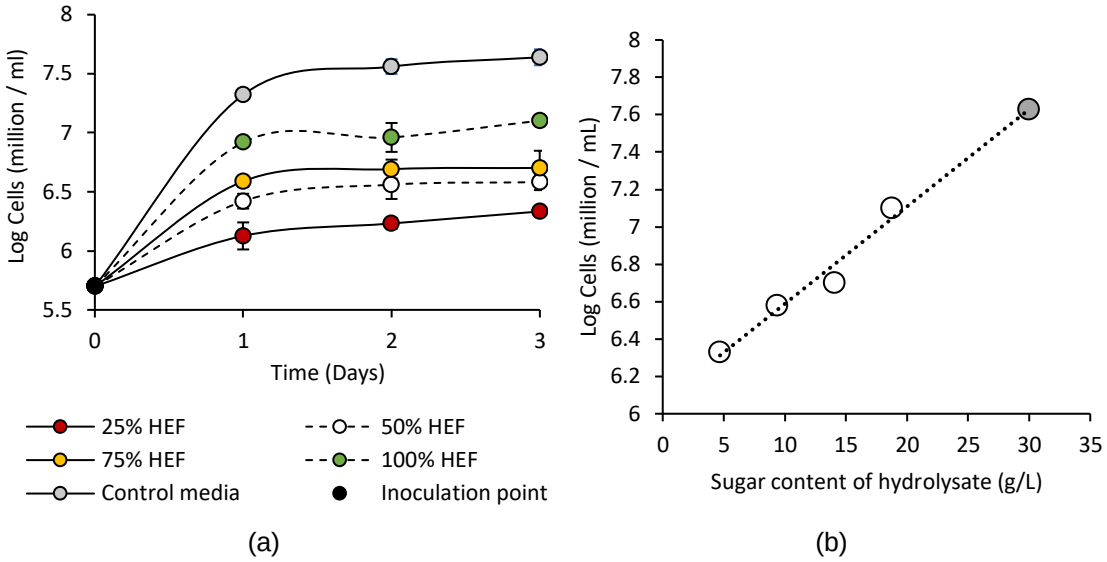


Figure 2-3 – Growth of Metschnikowia pulcherrima in HEF substrate obtained from the fermentation of SCG. Where a) is the logarithm of the number of cells per mL obtained over 3 days, and b) shows the final cell count as a function of the initial sugar loading in the hydrolysate.

2.2.5.2 Lignin enriched fraction (LEF)

LEF characterization

In the fractionation the lignin component is depolymerized into a thick bio-oil which is recovered from the MIBK. The main use for the LEF is as a biofuel component for combustion to be used to recover process energy on site or potentially to be used as a liquid fuel in a static combustion engine. As such, the high heating value (HHV) of this fraction is the most important features.

Initially elemental analysis was used to assess the LEF fraction (Table 2-3). This analysis not only helps to characterize these samples but can also be used in the calculation of the theoretical HHV. According to Ziomek and Williams, lignin is composed by approximately 63% of carbon, 5-6% of hydrogen, 0-1% of nitrogen and 1.5-3.5% of sulfur (32). In the samples obtained from the solvent systems MEW and MAW in Table 2-3, the carbon content is far lower than these values while the nitrogen content is higher. This may indicate the presence of protein in these samples. The samples obtained with MW show similar content to the one expected from lignin samples. This suggests that molecules such as protein and lipids must have migrated to the other fractions (CEF or HEF). However, the extremely low percentages of LEF obtained when using the MW solvent system suggest that most of the lignin present in both biomass sources may have also deposited in the CEF fraction. The experimental HHV was determined using a bomb

calorimeter while the theoretical HHV's were calculated using the percentages of elements obtained in the elemental analysis and Equations 2.1-2.3.

$$HHV\ [Btu/lb] = 145.44C + 620.28H + 40.5S - 77.54O \quad \text{Eq. 2.1}$$

$$HHV\ [Btu/lb] = 151.2C + 499.77H + 45S - 47.8O + 27N \quad \text{Eq. 2.2}$$

$$HHV\ [Btu/lb] = \left(\frac{654.3H}{100 - A} + 424.62\right) \times \left(\frac{C}{3} + H - \frac{O}{8} + \frac{S}{8}\right) \quad \text{Eq. 2.3}$$

Equations 2.1-2.3 are given in British thermal unit per pound which were then converted to Joule per gram to be in the same units as the value obtained from the bomb calorimeter (32-35). Note that C, H, S, O, N and A are the contents of carbon, hydrogen, sulphur, oxygen, nitrogen and ash in weight percentage. The value of ash was assumed to be null in Equation 2.3. The models show highly similar results to one another, although were not as accurate as predicting the calculated HHV values, with the largest discrepancies being observed for the SCG LEF fractions (Table 2-4). This suggests that in the LEF fraction of SCG there are numerous biomolecules present that simplistic models cannot account for. Additionally, the high error between the models and the experimental results for the MW samples indicates once more that this solvent system leads to fractions with higher percentage of impurities.

Table 2-3 – Elemental analysis results performed to LEF samples. The "total metals" column is a sum of all the metals present in the respective sample obtained from ICP-OES, and the "total" column is the sum of the elemental analysis and the "total metals" column.

Solvent	Biomass	Elemental Analysis (%)						
		C	H	N	S	O	Total metals	Total
MEW	Pist	31.54	3.64	1.35	0.28	14.65	46.15	97.61
	SCG	57.14	6.93	5.69	0.59	18.47	9.51	98.33
MAW	Pist	44.52	5.05	1.54	0.34	17.05	29.47	97.97
	SCG	61.84	8.56	2.57	0.81	16.03	8.60	98.41
MW	Pist	64.86	7.66	0.26	2.55	19.62	5.44	100.39
	SCG	62.82	9.17	0.74	3.81	21.13	0.15.	97.82

Table 2-4 – Experimental and theoretical high heating value (HHV) with respective errors to the experimental values.

Solvent	Biomass	HHV_{exp} [J/g]	Theoretical HHV (1)			Theoretical HHV (2)			Theoretical HHV (3)		
			Btu/lb	J/g	Error (%)	Btu/lb	J/g	Error (%)	Btu/lb	J/g	Error (%)
MEW	Pist	14,854	5,720	13,306	11.6	5,938	13,812	7.5	5,541	12,889	15.2
	SCG	22,038	11,201	26,053	15.4	11,402	26,521	16.9	11,158	25,953	15.1
MAW	Pist	19,339	8,299	19,304	0.2	8,499	19,768	2.2	8,147	18,950	2.1
	SCG	25,818	13,093	30,455	15.2	12,969	30,167	14.4	13,107	30,487	15.3
MW	Pist	10,792	12,767	29,695	63.7	12,821	29,822	63.8	12,887	29,976	64.0
	SCG	11,081	13,340	31,030	64.3	13,265	30,854	64.1	13,543	31,500	64.8

The HHV is substantially higher for the SCG components than for the pistachio hulls, and higher than is typically found for lignin components alone. Lignin is also a valuable source of aromatics, and as such could be used for further chemical production. The LEF samples were therefore analyzed by 2D NMR and GC-MS to understand the functionality present in the fraction.

All the spectra obtained in this analysis were compared with previous literature reports (29) (Figure 2-4 to Figure 2-7, Table 2-5). The 2D NMR demonstrates the large presence of aromatic species in the phase for all species examined. Furthermore, the presence of tricin and syringyl are the most prominent units in the pistachio hull, normally observed in woody biomass (36). In the SCG spectra for all fractions, the presence of *p*-coumarate and ferulates were observed, these are commonly found in food wastes (37). Additionally, carbohydrates were also observed in some of the samples, demonstrating some contamination from molecules that should have been deposited in one of the other fractions. Species common to both feedstocks include, alkyl-aryl groups, methoxyls, guaiacyl and p-hydroxyphenyl units, usually found in lignin. Finally, the samples obtained using MW solvent system showed higher number of unidentified peaks, when compared to the samples obtained using MEW and MAW.

Table 2-5 – Assignments to the labels in Figure 2-4 to Figure 2-6, see Figure 2-7 for the structures identified.

Label	Assignment
$A_{\alpha(G)}$	C_α-H_α in *β-O*-4'substructures (A) linked to a G-unit
A_γ	C_γ-H_γ in *γ*-hydroxylated *β-O*-4' substructures (A)
G_2	C_2-H_2 in guaiacyl units (G)
G_5/G_6	C_5-H_5 and C_6-H_6 in guaiacyl units (G)
$H_{2,6}$	$C_{2,6}$-$H_{2,6}$ in *p*-hydroxyphenyl units (H)
OMe	C-H in methoxyls
PCA_α	C_α-H_α in *p*-coumarate (PCA)
PCA_β/FA_β	C_β-H_β in *p*-coumarate (PCA) and ferulate (FA)
$S_{2,6}$	C_2-H_2 and C_6-H_6 in etherified syringyl units (S)
T_6/T_8	C_6 and C_8 in tricin (T)

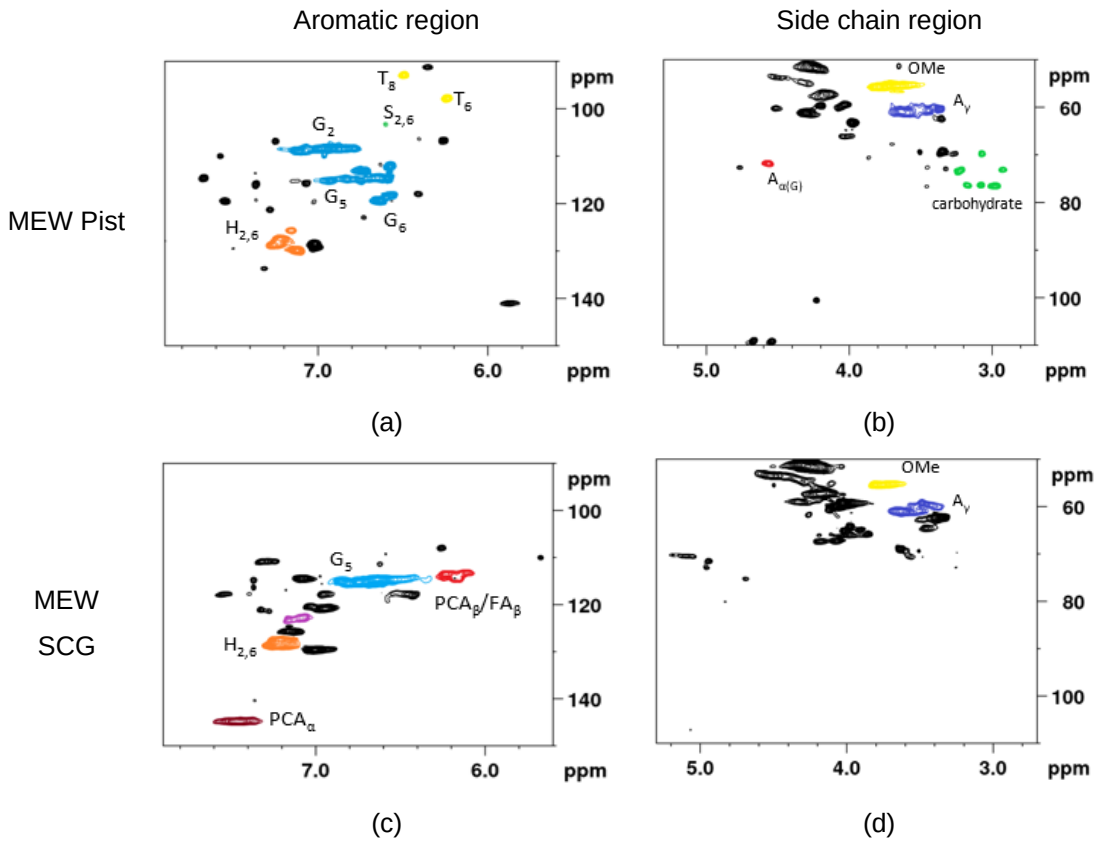


Figure 2-4 – 2D NMR analysis of LEF obtained using MEW. See Table 2-5 for label assignments.

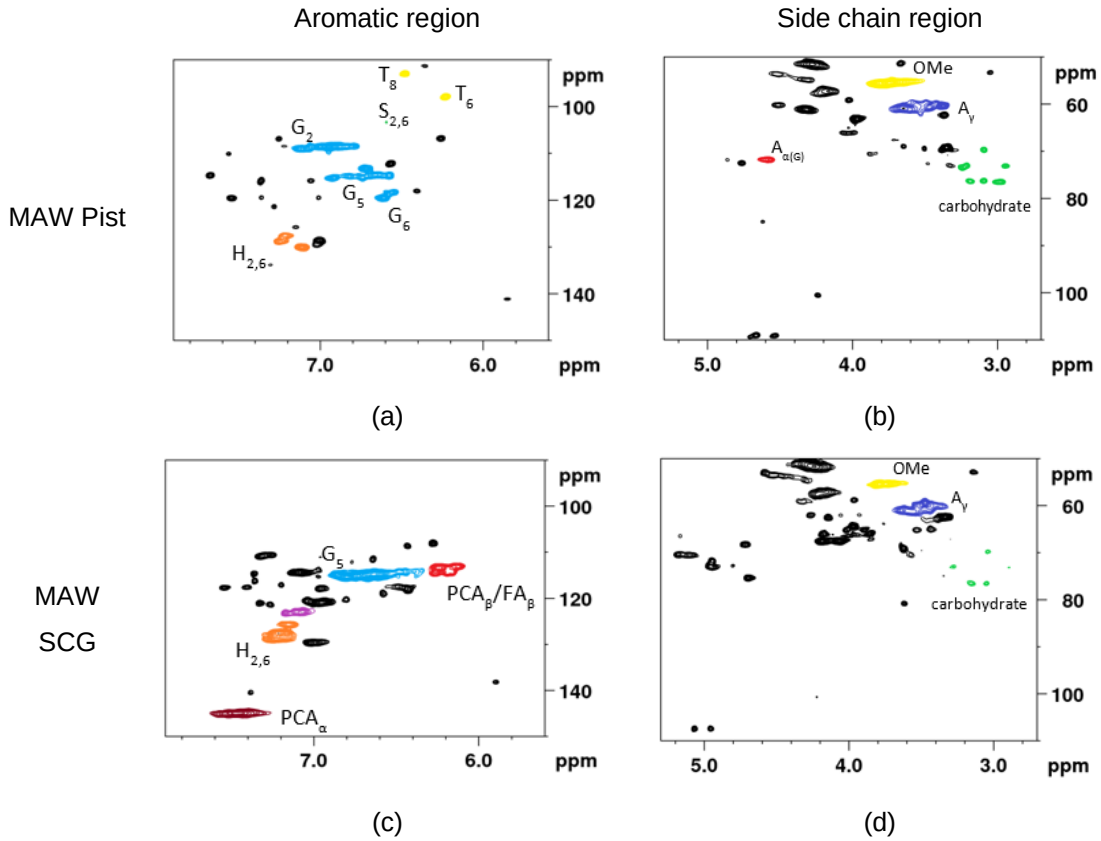


Figure 2-5 – 2D NMR analysis of LEF samples obtained using MAW. See Table 2-5 for label assignments.

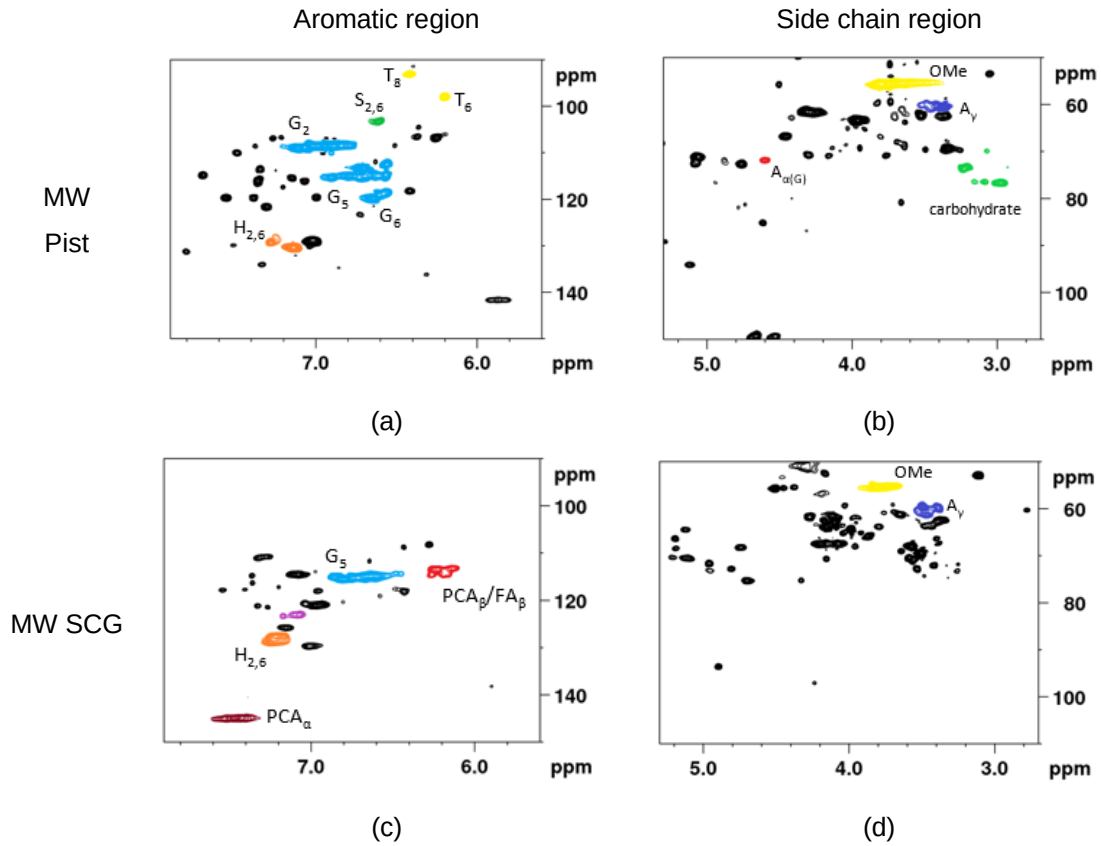


Figure 2-6 – 2D NMR analysis of LEF samples obtained using MW. See Table 2-5 for label assignments.

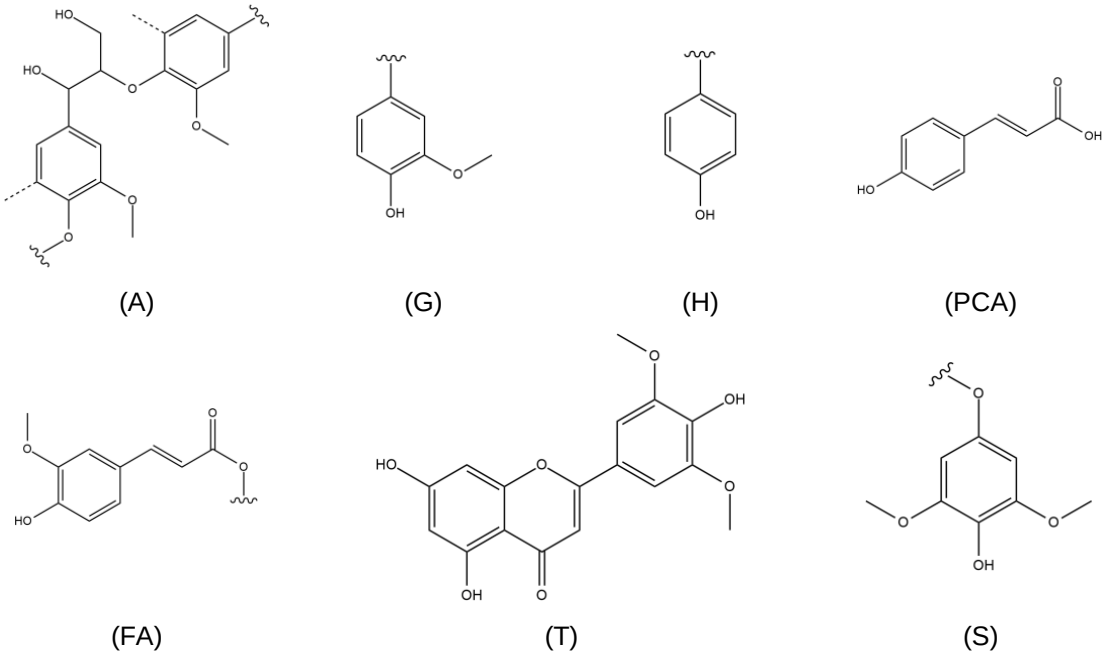


Figure 2-7 - Main structures identified using 2D NMR: (A) β-O-4' alkyl-aryl ethers with acylated γ-OH; (G) guaiacyl unit; (H) p-hydroxyphenyl unit; (PCA) p-coumarates; (FA) ferulates; (T) tricin; (S) syringyl unit.

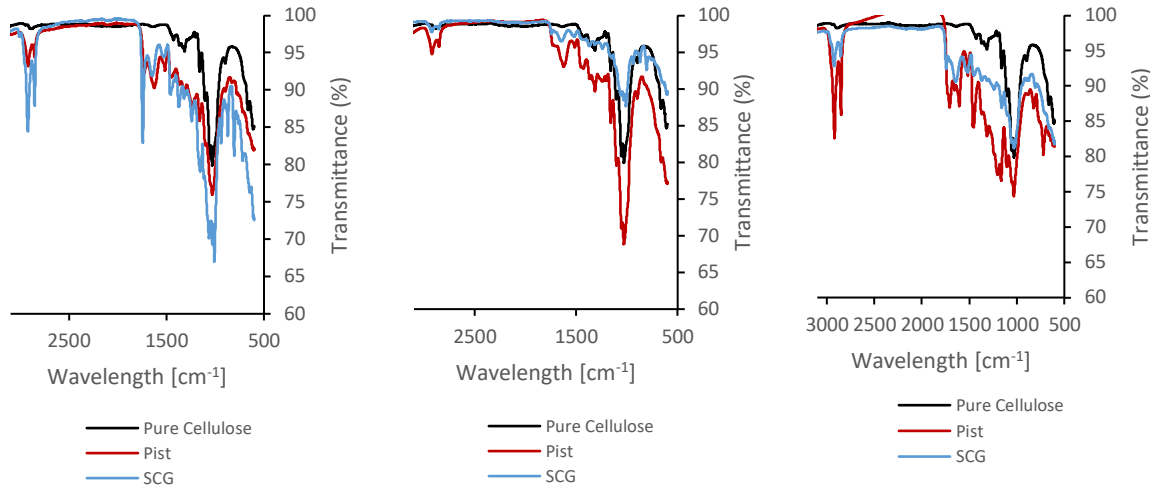
GC-MS was also used to identify the LEF fraction (see Appendix A, Figure 2-11 to Figure 2-16); this analysis confirmed the presence of smaller lignin units, and of fatty acids, which are produced by the hydrolysis of triglycerides in the coffee oil. This would account for the higher HHV of the SCG samples. This confirms that the major two components in this phase are the lipids and lignin fragments from the SCG.

2.2.5.3 Cellulose enriched fraction (CEF)

CEF characterization

CEF was characterized using four types of solid-state analysis: Fourier-transform infrared spectroscopy (FT-IR), solid state NMR (ssNMR), elemental analysis and thermogravimetric analysis (TGA). For FT-IR, the elemental analysis and TGA, the CEF samples were compared to pure cellulose.

In the FT-IR analysis, the samples were divided according to the solvent system, allowing the comparison between pure cellulose and CEF from pistachio hull and SCG (Figure 2-8). A large clear peak around 1000-1100 cm^{-1} is observable in all the spectra. This peak is characteristic of a C-O bond in the glucose monomer in cellulose. Additionally, another peak was observed around 3000 cm^{-1}; characteristic of an alkyl group from the glucose ring was observed for all the fractions.



(a) MEW (b) MAW (c) MW

Figure 2-8 – Fourier-transform infrared spectroscopy (FT-IR) spectra of the CEF samples

All the CEF fractions that were obtained from pistachio and SCG differed depending on the solvent system used. While distinctive peaks were observed for CEF in the MEW extraction, there were a number of other peaks suggesting esters and possibly proteinous species in both the MEW solid fractions derived from the pistachio and SCG compounds. The cellulose fraction isolated using MAW was the most similar to pure cellulose with few other species observable for the SCG other than those for cellulose. The spectra relating to the pistachio CEF were highly similar for both MEW and MAW solvent extraction. The CEF samples obtained using MW, pistachio in particular, show some more unidentified peaks between 1200 and 1800 cm^{-1}. Once more, this may be an indicator that the fractionation using this solvent led to samples with higher level of impurities. This correlates with the purity of the LEF fraction in the MAW samples and demonstrates the poor fractionation potential of the MW system.

To assess the samples in more detail, the solids were analyzed by solid state NMR (ssNMR). A ^{13}C and ^{1}H spectra were obtained for each sample (Figure 2-9). In the ^{13}C spectra for the pistachio CEF, peaks characteristic of cellulose were observed between 60-100 ppm, although further downfield peaks assignable to acid or ester linkages were also present. The proton spectrum showed a broad, unresolved peak, which is typical of amorphous solid materials in this type of analysis. The ^{13}C and ^{1}H spectra for the SCG CEF were far better resolved. These narrower peaks are either associated with highly crystalline material, such as cellulose or a “soft” (mobile) component. Both of these spectra demonstrate a high level of cellulose has been extracted but that it is heavily contaminated with non-aromatic components such as fatty acids/esters or amino

acid/proteinous material. The elemental analysis of the CEF fraction confirms this likely contamination to be protein for both the SCG and pistachio (Table 2-6). Compared to pure cellulose all the CEF fractions had elevated carbon, N and S while have lower O.

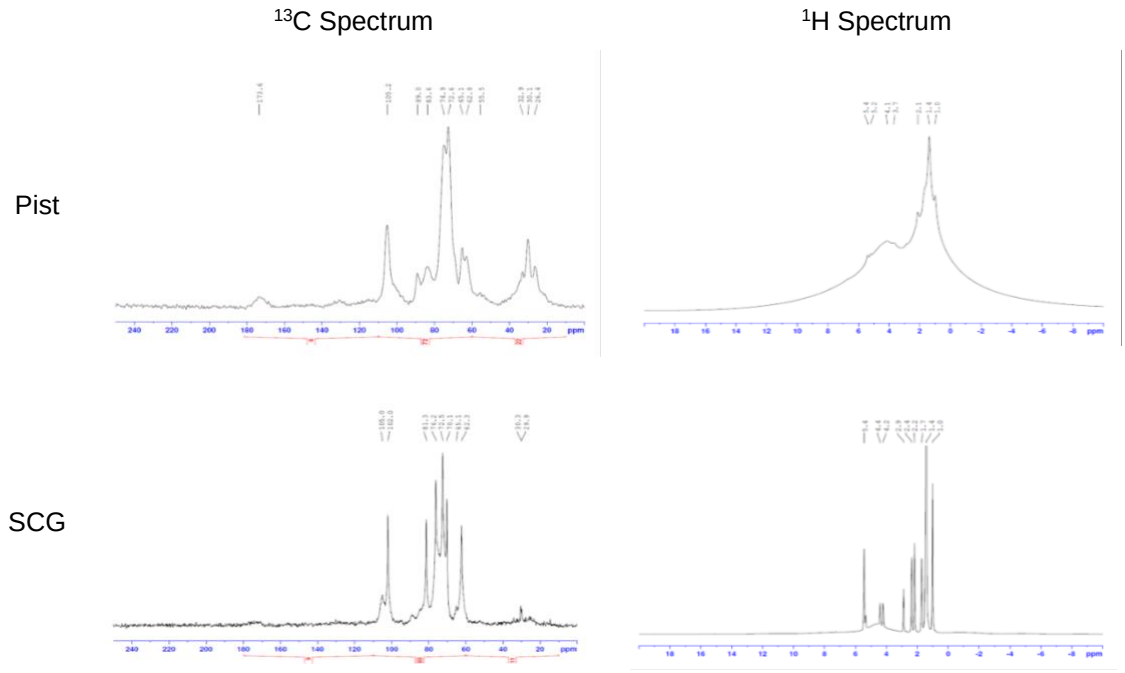


Figure 2-9 – Solid state Nuclear Magnetic Resonance (ssNMR) ^{13}C, ^{1}H spectra for MEW samples.

Table 2-6 – Elemental analysis of CEF. The column denominated "Total" corresponds to the sum of the analyzed elements.

		C (%)	H (%)	N (%)	S (%)	O (%)	Total (%)
Cellulose		**44.40**	**6.20**	**0.00**	**0.00**	**49.40**	**100.00**
MEW	Pist	50.36	6.82	1.38	0.28	36.52	95.36
	SCG	52.02	7.64	1.38	0.31	36.40	97.75
MAW	Pist	52.81	7.17	1.38	0.38	35.80	97.54
	SCG	49.38	7.28	1.65	0.30	39.51	98.12
MW	Pist	58.76	7.82	1.23	0.67	30.46	98.94
	SCG	56.11	7.74	3.23	0.46	30.45	97.99

To estimate the amount of cellulose in the samples, thermogravimetric analysis was performed on all the six samples of CEF (Figure 2-10). By comparing the reduction in weight in the sample to that of pure cellulose, the total cellulose in the samples can be estimated (Table 2-7). The percentage of cellulose in the CEF fractions produced from pistachio hulls is higher than CEF from SCG, irrespective of the solvent system used. This is presumably due to the higher level of cellulose and lower protein and other

nitrogen species in the hulls. Additionally, the lower percentage of cellulose in the MW fraction demonstrates the poor separation afforded by the MW system.

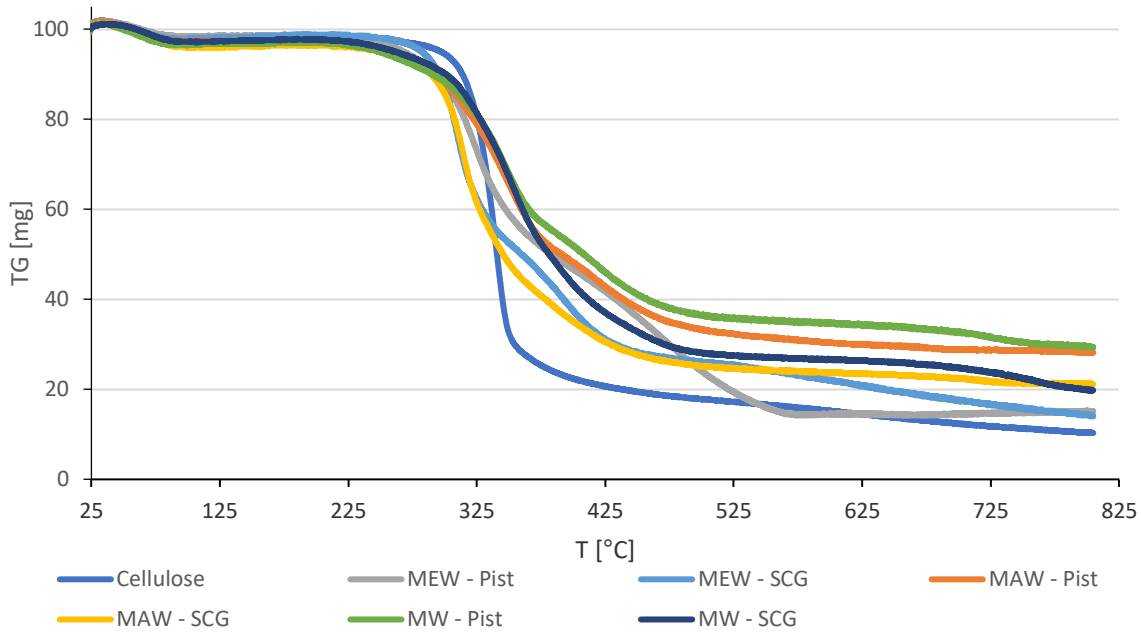


Figure 2-10 – Thermogravimetric analysis (TGA) of CEF samples from pistachio hull and SCG.

Table 2-7 – Percentage of cellulose and ash in CEF samples based on TGA

Solvent	Biomass	Estimated $\%_{cellulose}$
MEW	Pist	43.7
	SCG	41.0
MAW	Pist	45.5
	SCG	37.0
MW	Pist	36.6
	SCG	35.5

Production of HMF from CEF

To demonstrate the suitability of the CEF fraction for HMF production, each fraction was converted without any further purification. This was then compared to the same sample being enzymatically hydrolyzed to glucose before being converted to HMF and finally to the glucose being isomerized to fructose and this converted to HMF (Table 2-8). Each of the CEF fractions were compared with the same procedure for pure cellulose. The initial production of HMF directly from CEF yielded very low conversions for all systems, including pure cellulose. This is expected and is due to the high complexity, crystallinity and supramolecular structure of the cellulose (38). Glucose is less stable, soluble and

as such higher conversions can be obtained. A 61% yield of glucose was achieved from cellulose, although, due to the high stability of the pyranoside ring structure which has previously been reported to be a limiting factor to achieve high HMF yields (39), only 24% of the pure cellulose material was converted to HMF. This is a yield of 14.8% of the original starting material.

A far lower glucose yield was observed for both the CEF for pistachio (24%) and SCG (26%). However, when the reduced amount of cellulose of the CEF is factored in, then this is a similar yield to cellulose, suggesting that the CEF is not inhibitory to enzymes.

The most suitable starting material for HMF production is fructose (30). Therefore, all glucose streams were isomerized to this sugar. The HMF yields from fructose were far higher, with just under 80% of the sugar converted to HMF; this result is similar to previous reports (30,40). The glucose produced from both CEF fractions could be isomerized yielding similar amounts of fructose, on conversion 69.6% HMF was produced from pistachio and 70.3% from SCG. This gave a final yield of 8.2% from the original starting material for SCG and 8.0% from pistachio.

Table 2-8 – Summary of the cellulose/CEF hydrolysis, glucose isomerization and fructose dehydration

Biomass	Glucose Yield (%)	Fructose Yield (%)	HMF Yield (%)	Overall HMF Yield (%)
Cellulose	-	-	0.8	0.8
	60.8	-	24.3	14.8
	60.8	52.2	73.2	23.2
CEF from Pistachio	-	-	0.1	0.1
	23.7	-	21.8	5.2
	23.7	48.3	69.6	8.0
CEF from SCG	-	-	0.1	0.1
	25.9	-	22.0	5.7
	25.9	44.9	70.3	8.2

2.2.6 Discussion

The similar masses obtained in the fractionation process for SCG and pistachio hull indicate that this process, initially developed for lignocellulosic biomass sources, can be efficiently used in the separation of the components present in SCG. The CEF mass yields obtained are approximately in accordance with the higher content of cellulose present in pistachio hull than in SCG (14% and 12%, respectively). Both the MAW and MEW solvent systems also deposit a range of other components, up to 60%, into this

phase. Though this is in accordance with other lignocellulosic biomass separated through this method (28).

The poor performance of the MW system demonstrates how both ethanol and acetone aid in the fractionation process. When these solvents are not used most of the lignin remains as a solid species and is deposited elsewhere in the system. This reduces the ability to used LEF for bioenergy. Despite of the better fractionation performance, the presence of ethanol and acetone in the HEF fraction, demonstrated by the increased TOC in the phase, make it unsuitable for fermentation. In contrast the aqueous fraction produced with MW contained all the micronutrients necessary for growth and could be used to grow the oleaginous yeast *M. pulcherrima*; interestingly the growth of the yeast was only limited by the sugar present and not inhibited by the presence of anything in the broth. For all the samples, the HEF fraction had a higher total nitrogen content when SCG was used. While this will relate to bioactive compounds such as caffeine as well as the increased protein content, it did not have an inhibitory effect on the yeast.

The sugar content of the HEF fraction shows that while HEF from pistachio hull can be used in the production of furfural, the absence of C_5 sugars in SCG means that furfural could not be produced as a co-product with HMF in a SCG biorefinery. Instead, the presence of C_6 sugars, such as galactose and mannose indicate that this fraction can be used in the production of HMF, although with similar issues associated with the conversion of glucose. While the sugars in the HEF fraction could be fermented, to achieve a good fractionation in the overall system, ethanol and acetone must be used – this severely limits the use of these sugars without further costly separations.

The LEF fraction, solubilized in MIBK, obtained using MEW and MAW had a reasonably high HHV (15-26 MJ/kg) when compared to the LEF fraction from MW (approximately 11 MJ/kg). For both the MEW and MAW solvent systems, the LEF fractions from SCG had higher HHV (22-26 MJ/g) than pistachio hull (15-19 MJ/kg). This suggests that this fraction of SCG has higher potential to be used as a biofuel component. However, both the GC-MS and 2D NMR analysis of this fraction demonstrated the presence of depolymerized lignin and fatty acids. Both materials have elevated lipids, though the SCG has more. This indicates that this fraction may also be used in the production of renewable biodiesel and polymers, however similar to the HEF fraction, the fatty acids would need to be separated from the other components, which limit the applicability for lower value uses. One possibility would be to extract the lipids prior to the fractionation.

The final fraction contains the solids left over from the process, predominantly cellulose (CEF). The elemental analysis, TGA, ssNMR and FT-IR demonstrate that cellulose is

present in both samples, up to approximately 40% of the phase. A large proportion of nitrogen and sulfur were also observed suggesting that protein was present, although there was no clear indication of high levels of lignin in this phase. As with the other phases the CEF fraction from the MW separation was heavily contaminated for both biomass sources tested. The CEF fraction can be depolymerized to release glucose for either fermentation or further chemical manufacture. For the manufacture of HMF, the cellulose is too stable, and little HMF was produced from any of the CEF fractions. Indeed, it is only the conversion through multiple enzymatic steps to fructose that yielded reasonable HMF concentrations. While only 8.2% of the original starting material of HMF was produced from the SCG, 8.0% HMF was produced from pistachio hull. This is partly due to low percentage of cellulose in CEF and the thermodynamic equilibrium between glucose and fructose which limits the reactions yields (41). According to Al-Tai *et al.* the maximum theoretical yield that can be obtained in the glucose isomerization reaction is approximately 50% (42). This was the limiting step and reduces amounts of HMF produced per batch. As such, the yields obtained from SCG and pistachio were highly similar to crystalline cellulose when the lower amount of cellulose was taken into account. This suggests that the fractionation works well, and that there are no inhibitory compounds in this phase for further processing.

Considering the entire process of biomass fractionation (using both pistachio and SCG) and the subsequent conversion of CEF to HMF, it was possible to produce approximately 0.37 g of HMF from 10 g of pistachio hull and approximately 0.35 g from SCG. This value of approximately 4% is close to the theoretical possible, considering that there is 14% and 12% of cellulose in pistachio hull and SCG, respectively, that the conversion of cellulose to glucose is limited to between 75-95%, the conversion of glucose to fructose is limited by the kinetic equilibrium between these two sugars, which is translated to a maximum theoretical yield obtained is 50% and maximum HMF yield from fructose is 80%.

2.2.7 Conclusions

In the paper a biorefinery based around the organosolv fractionation of spent coffee grounds (SCG) was attempted for the first time. The fractionation of SCG led to similar results to the fractionation of a lignocellulosic material, in this case pistachio hull. These results demonstrate that this organosolv process can effectively separate the components present in SCG into three different enriched fractions: yielding a solubilized fraction predominantly from hemicellulose (HEF), a cellulose enriched solid (CEF) and a lignin enriched organic phase (LEF). The fractionation worked well when ethanol and acetone were used, though both of these solvents partitioned into the aqueous phase,

inhibiting the use as a fermentation media. On fractionating with just water and MIBK, the HEF fraction could be used for fermentation and was only limited by the amount of sugars that partitioned there. For the SCG this was predominantly C_6 mono- and disaccharides.

The LEF samples for the SCG had very high HHV and were predominantly made up of lignin fragments and fatty acids. Both the MEW and MAW solvent systems were suitable for this fractionation. Finally, the CEF fraction was analyzed and demonstrated to contain large content of cellulose alongside a range of other macromolecules. The CEF fraction was shown to be suitable for the production of HMF, with close to the theoretical yield obtained when depolymerized to glucose and isomerized to fructose prior to conversion. This paper demonstrates that the organosolv process is suitable for fractionating SCG and multiple products can be produced in a biorefinery concept.

2.2.8 Acknowledgments

We gratefully acknowledge the solid-state NMR group at Durham University for their help in running the CEF fraction samples. Solution state NMR spectra were acquired using instruments from the Materials and Chemical Characterisation facility at the University of Bath.

2.2.9 Appendix A

2.2.9.1 Chromatograms from gas chromatography mass spectrometry

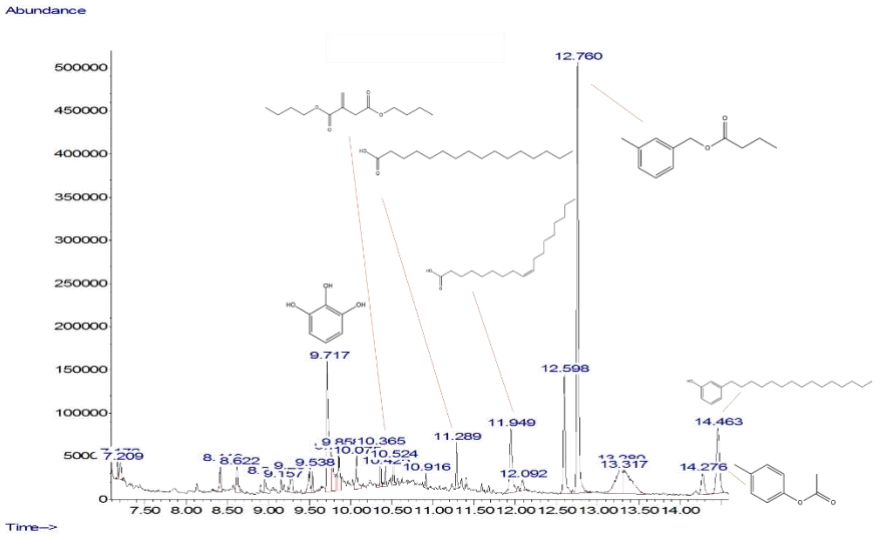


Figure 2-11 – GC-MS chromatogram obtained from MEW – pistachio hull

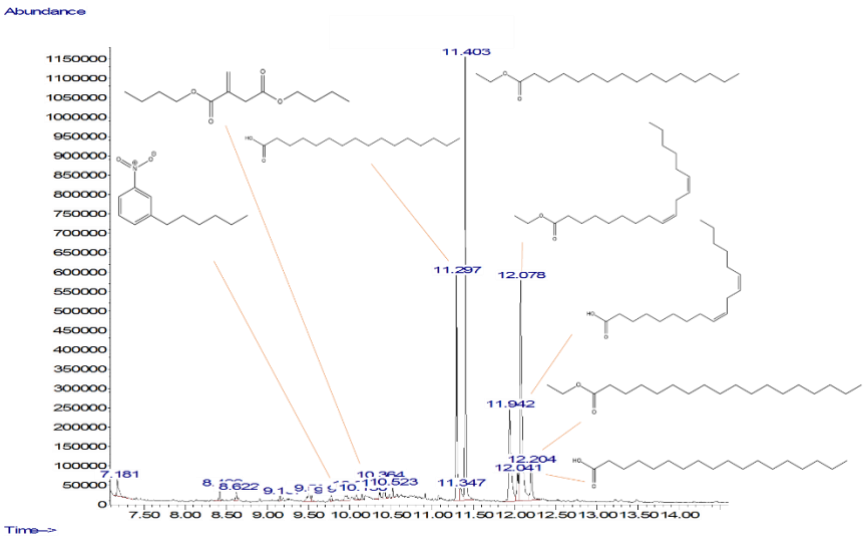


Figure 2-12 – GC-MS chromatogram obtained from MEW – SCG

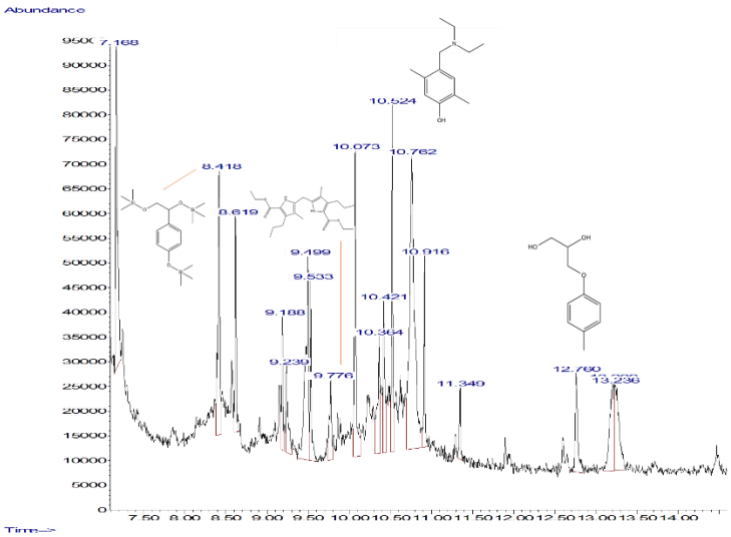


Figure 2-13 – GC-MS chromatogram from MAW – pistachio hull

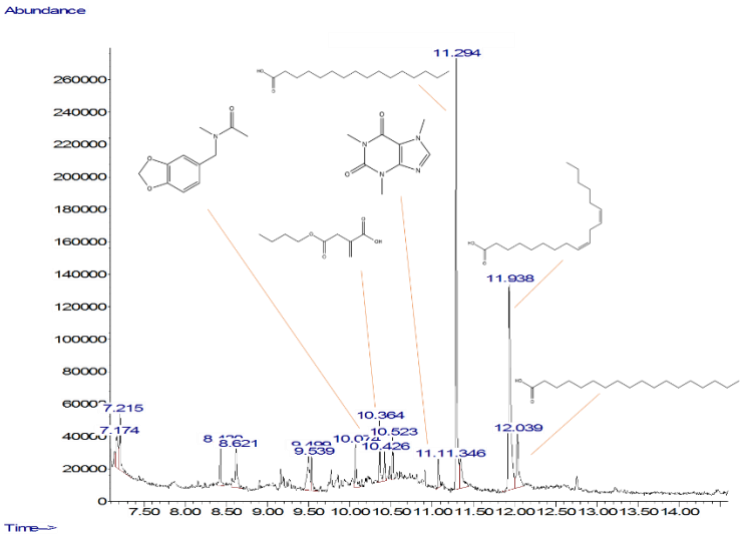


Figure 2-14 – GC-MS chromatogram from MAW – SCG

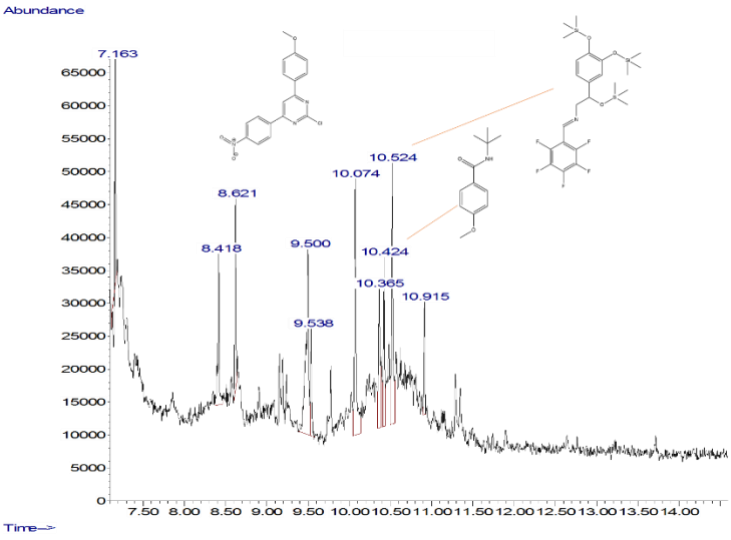


Figure 2-15 – GC-MS chromatogram from MW – pistachio hull

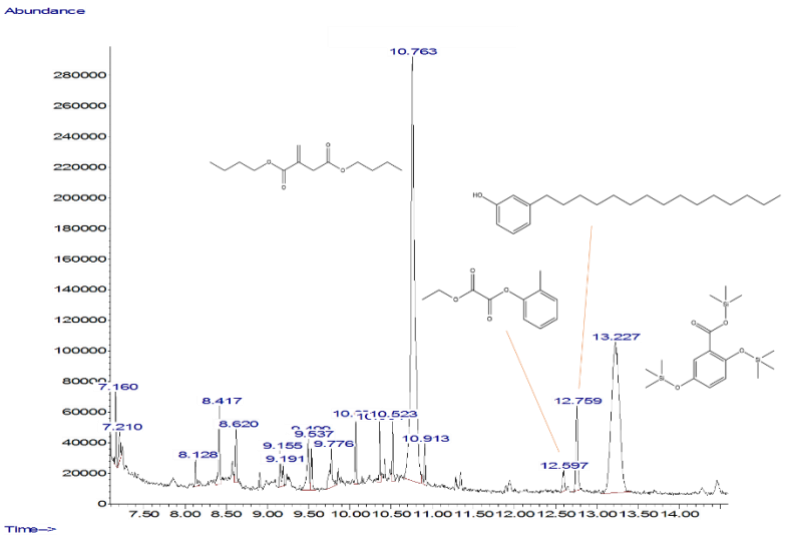


Figure 2-16 – GC-MS chromatogram from MW – SCG

2.2.9.2 Pistachio hull content

Table 2-9 – Analysis of pistachio hulls used in this study.

Sample description	% Total Ash	% Structural Inorganics	% Non-Structural Inorganics	% Total Protein	% Structural Protein	% Non-Structural Protein	Soluble sugars												
							% Sucrose	% Free Glucose	% Free Fructose	% Water Extractable Others	% Ethanol Extractives	% Lignin	% Glucan	% Xylan	% Galactan	% Arabinan	% Fructan	% Acetyl	Total %
Pistachio hull	9.4	5.4	4.0	7.5	6.2	1.3	0.0	0.0	0.0	15.6	11.1	26.6	13.6	5.0	2.8	4.8	0.0	1.4	91.3

2.2.10 References

1. Baeza G, Sarriá B, Bravo L, Mateos R. Polyphenol content, in vitro bioaccessibility and antioxidant capacity of widely consumed beverages. J Sci Food Agric. 2017;98(4):1397–406.
2. United States Department of Agriculture. Coffee: World markets and Trade. 2018.
3. United States Department of Agriculture. Coffee: World markets and trade. 2012.
4. United States Department of Agriculture. Coffee: World markets and Trade. 2015.
5. Cunha MR da. Apêndice estatistico. BACHA, E GREENHILL. 1992;286–388.
6. Mussatto SI, Machado EMS, Martins S, Teixeira JA. Production, Composition, and Application of Coffee and Its Industrial Residues. Food Bioprocess Technol. 2011 Mar;4(5):661.
7. Massaya J, Prates-Pereira A, Mills-Lamptey B, Benjamin J, Chuck CJ. Conceptualization of a spent coffee grounds biorefinery: a review of existing valorisation approaches. Food Bioprod Process. 2019;in press.
8. Cruz R, Cardoso MM, Fernandes L, Oliveira M, Mendes E, Baptista P, et al. Espresso Coffee Residues: A Valuable Source of Unextracted Compounds. J Agric Food Chem. 2012;60(32):7777–84.
9. Ballesteros LF, Teixeira JA, Mussatto SI. Chemical, Functional, and Structural Properties of Spent Coffee Grounds and Coffee Silverskin. Food Bioprocess Technol. 2014 Dec;7(12):3493–503.
10. Karmee SK. A spent coffee grounds based biorefinery for the production of biofuels, biopolymers, antioxidants and biocomposites. Waste Manag. 2017;
11. Somnuk K, Eawlex P, Prateepchaikul G. Optimization of coffee oil extraction from spent coffee grounds using four solvents and prototype-scale extraction using circulation process. Agric Nat Resour. Elsevier; 2017 Jun 1;51(3):181–9.
12. Martinez-Saez N, García AT, Pérez ID, Rebollo-Hernanz M, Mesías M, Morales FJ, et al. Use of spent coffee grounds as food ingredient in bakery products. Food Chem. Elsevier; 2017 Feb 1;216:114–22.
13. Silva MA, Nebra SA, Machado Silva MJ, Sanchez CG. The use of biomass residues in the Brazilian soluble coffee industry. Biomass and Bioenergy. 1998;14:457–67.
14. Girotto F, Pivato A, Cossu R, Nkeng GE, Lavagnolo MC. The broad spectrum of

possibilities for spent coffee grounds valorisation. J Mater Cycles Waste Manag. 2017 Apr;

15. Acevedo F, Rubilar M, Scheuermann E, Cancino B, Uquiche E, Garces M, et al. Spent Coffee Grounds as a Renewable Source of Bioactive Compounds. J Biobased Mater Bioenergy. 2013;7:420–8.

16. Peshev D, Mitev D, Peeva L, Peev G. Valorization of spent coffee grounds – A new approach. Sep Purif Technol. 2018;192(Supplement C):271–7.

17. Kondamudi N, Mohapatra SK, Misra M. Spent Coffee Grounds as a Versatile Source of Green Energy. J Agric Food Chem. 2008;56(24):11757–60.

18. Jenkins RW, Stageman NE, Fortune CM, Chuck CJ. Effect of the Type of Bean, Processing, and Geographical Location on the Biodiesel Produced from Waste Coffee Grounds. Energy & Fuels. 2014;28(2):1166–74.

19. Liu Y, Tu Q, Knothe G, Lu M. Direct transesterification of spent coffee grounds for biodiesel production. Fuel. 2017;199:157–61.

20. Mussatto SI, Machado EMS, Carneiro LM, Teixeira JA. Sugars metabolism and ethanol production by different yeast strains from coffee industry wastes hydrolysates. Appl Energy. Elsevier; 2012 Apr 1;92:763–8.

21. Burniol-Figols A, Cenian K, Skiadas I V., Gavala HN. Integration of chlorogenic acid recovery and bioethanol production from spent coffee grounds. Biochem Eng J. Elsevier; 2016 Dec 15;116:54–64.

22. Caetano NS, Mata TM, Martins AA, Felgueiras MC. New Trends in Energy Production and Utilization. Energy Procedia. Elsevier; 2017 Feb 1;107:7–14.

23. Mata TM, Martins AA, Caetano NS. Bio-refinery approach for spent coffee grounds valorization. Bioresour Technol. Elsevier; 2018 Jan 1;247:1077–84.

24. Agarwal B, Kailasam K, Sangwan RS, Elumalai S. Traversing the history of solid catalysts for heterogeneous synthesis of 5-hydroxymethylfurfural from carbohydrate sugars: A review. Renew Sustain Energy Rev. Pergamon; 2018 Feb;82:2408–25.

25. Roman-Leshkov Y, J Barrett C, Y Liu Z, A Dumesic J. Production of Dimethylfuran for Liquid Fuels from Biomass-Derived Carbohydrates. Nature. 2007;447:982–5.

26. Kucherov FA, Gordeev EG, Kashin AS, Ananikov VP. Three-Dimensional Printing with Biomass-Derived PEF for Carbon-Neutral Manufacturing. Angew Chemie Int Ed. 2017;56(50):15931–5.

27. Bozell JJ, Black SK, Myers M, Cahill D, Miller WP, Park S. Solvent fractionation of renewable woody feedstocks: Organosolv generation of biorefinery process streams for the production of biobased chemicals. Biomass and Bioenergy. Pergamon; 2011 Oct 15;35(10):4197–208.

28. Katahira R, Mittal A, McKinney K, Ciesielski PN, Donohoe BS, Black SK, et al. Evaluation of Clean Fractionation Pretreatment for the Production of Renewable Fuels and Chemicals from Corn Stover. ACS Sustain Chem Eng. 2014;2(6):1364–76.

29. del Río JC, Rencoret J, Prinsen P, Martínez ÁT, Ralph J, Gutiérrez A. Structural Characterization of Wheat Straw Lignin as Revealed by Analytical Pyrolysis, 2D-NMR, and Reductive Cleavage Methods. J Agric Food Chem. 2012;60(23):5922–35.

30. Gallo JMR, Alonso DM, Mellmer MA, Dumesic JA. Production and upgrading of 5-hydroxymethylfurfural using heterogeneous catalysts and biomass-derived solvents. Green Chem. The Royal Society of Chemistry; 2013;15(1):85–90.

31. Santomauro F, Whiffin FM, Scott RJ, Chuck CJ. Low-cost lipid production by an oleaginous yeast cultured in non-sterile conditions using model waste resources. Biotechnol Biofuels. 2014;7(34):1–11.

32. Ziomek E, E Williams R. Modification of Lignins by Growing Cells of the Sulfate-Reducing Anaerobe Desulfovibrio desulfuricans. Appl Environ Microbiol. 1989;55:2262–6.

33. Selvig WA, Gibson FH. Calorific Value of Coal. In: Chemistry of Coal Utilization. 1945.

34. Boie W. Fuel Technology Calculations. Energietechnik. 1953;

35. Grummel ES, Davis IA. A new Method of Calculating the Calorific Value of a Fuel From Its Ultimate Analysis. Fuel1. 1933;

36. Lan W, Rencoret J, Lu F, Karlen SD, Smith BG, Harris PJ, et al. Tricin-lignins: occurrence and quantitation of tricin in relation to phylogeny. Plant J. 2016;88(6):1046–57.

37. Research W. FooDB [Internet]. 2018 [cited 2018 Nov 25]. Available from: http://foodb.ca/compounds/FDB002595

38. Su Y, Brown HM, Huang X, Zhou X, Amonette JE, Zhang ZC. Single-step conversion of cellulose to 5-hydroxymethylfurfural (HMF), a versatile platform

chemical. Appl Catal A Gen. Elsevier; 2009 Jun 20;361(1–2):117–22.

39. Hu L, Zhao G, Hao W, Tang X, Sun Y, Lin L, et al. Catalytic conversion of biomass-derived carbohydrates into fuels and chemicals via furanic aldehydes. RSC Adv. The Royal Society of Chemistry; 2012;2(30):11184–206.

40. Chheda J, A. Dumesic J. Production of 5-hydroxymethylfurfural and furfural by dehydration of biomass-derived mono- and poly-saccharides. Green Chem - GREEN CHEM. 2007;9.

41. Moliner M, Román-Leshkov Y, Davis ME. Tin-containing zeolites are highly active catalysts for the isomerization of glucose in water. Proc Natl Acad Sci. National Academy of Sciences; 2010;107(14):6164–8.

42. Al-Tai AM, Ali Y, Abdul-Razzak SH. Isomerization of glucose to fructose: production and some properties of glucose isomerase from Streptomyces sp. strain C7. Enzyme Microb Technol. Elsevier; 1987 Oct 1;9(10):632–4.

Chapter 3

An alternative biorefinery approach to address microalgal seasonality: blending with spent coffee grounds

3.1 Context

Microalgal based integrated biorefineries have been gaining increased attention in recent years. This is due to the relatively straightforward production of microalgae which is considerably higher than the terrestrial biomass type growth, can be grown in non-arable lands and therefore does not compete with food crops for arable land, and the lack of requirement for potable water in the microalgae growth. In addition, microalgae also have a biomolecular rich composition in carbohydrates, lipids and protein. Such composition allows for the production of a wide range of biofuels and the repurpose of waste streams in the biorefinery to produce further value-added products.

However, one of the main challenges with microalgal-based biorefineries is the seasonality of microalgae. The lower production and therefore, availability of microalgae in certain seasons of the year poses a problem to the biorefinery operating levels. One solution could be the reduction of the operating levels to match the lowest production of microalgae. However, this solution would drastically reduce the product quantities during the lower season, as microalgae production can drop down to 40% biomass yield in these times. An alternative solution could be the storage of the microalgae during periods of higher supply to be used in periods of lower availability. However, elevated capital costs, degradation of the microalgae and overall loss of material have been shown.

To this end, this study aimed to blend the microalgae with spent coffee grounds (SCG) in periods of lower availability of microalgae. Just like microalgae, SCG also have a diverse biomolecular composition in carbohydrates, lipids and protein. Two blends of microalgae and SCG were studied. The first blend with 40% of microalgae and 60% of SCG simulates the lower production of microalgae season, while the second blend with 60% of microalgae and 40% of SCG simulates the intermediate seasons. These blends were tested in parallel with both pure feedstocks in a Combined Algal Processing (CAP) biorefinery approach developed at the National Renewable Energy Laboratory (NREL) designed to produce bioethanol and extract the lipids. In addition to this biorefinery design and evoking the concept of integrated biorefinery, a hydrothermal liquefaction (HTL) was performed on the solid waste stream from this design to produce bio-crude and biochar.

This chapter is submitted in an alternative format in line with Appendix 6A of the “Specifications for Higher Degree Theses and Portfolios” as required by the University of Bath.

All the experimental work in this chapter was undertaken by the author with the exception of the following:

Acid pretreatment was carried out by Nick Nagle, a co-author in this paper.

Analytical work was performed by Bonnie Panczak, a co-author in this paper.

The full journal reference is:

Pereira, Andre Prates, Tao Dong, Eric P. Knoshaug, Nick Nagle, Ryan Spiller, Bonnie Panczak, Christopher J. Chuck, and Philip T. Pienkos. "An alternative biorefinery approach to address microalgal seasonality: blending with spent coffee grounds." Sustainable Energy & Fuels (2020).

3.2 Sustainable Energy and Fuels paper

An Alternative biorefinery approach to address microalgal seasonality: blending with spent coffee grounds

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3.2.1 Key words

Biorefinery

Microalgae

Microalgae seasonality

Spent Coffee Grounds

Biofuels

3.2.2 Abstract

An effective method for the production of fuels and chemicals from microalgae is to ferment the carbohydrate fraction, extract the lipids and convert the resulting solids through hydrothermal liquefaction (HTL). In this process, known as Combined Algal Processing (CAP), multiple fuel precursors are produced effectively. However, one of the key challenges associated with a microalgae-based biorefinery is the reduced productivity of algae in the colder seasons. In this investigation, the potential for spent coffee grounds (SCG), a potentially valuable waste stream, to be blended with biomass from the microalgae Scenedesmus acutus (HCSD) to make up for the productivity shortfalls in periods of lower microalgae productivity to maximize the capacity for downstream equipment throughout the year was evaluated. Two different blend ratios were compared to only microalgae biomass or SCG, one representing winter season (40% microalgae and 60% SCG – blend 1) and another representing autumn and early spring (60% microalgae and 40% SCG – blend 2). Pretreatment of the blends showed higher monosaccharide release yields compared to microalgae alone, with an increase in mannose and galactose specifically. In the fermentation of the pretreated slurries, all the monosaccharides were consumed, resulting in ethanol titers of up to 23 g/L for the SCG blend, compared to 14 g/L ethanol for the algae alone. The lipid extraction from the blends resulted in yields of 95.5-99.7% (which translates to 173.8-193.5 kg/tonne of dry biomass processed in this biorefinery scenario) compared to 92.2% in HCSD (216.2 kg/tonne of dry biomass) and 68.1% in SCG (90.8 kg/tonne of dry biomass) alone. The residual solids left after fermentation and lipid extraction were converted *via* hydrothermal liquefaction (HTL) to produce bio-crude. The bio-crude yield was higher for microalgae (24.6%) than for the two blend cases (blend 1 - 17.5% and blend 2 - 19.7%). Theoretical energy calculations showed that the addition of SCG gave similar yields of fuel (gallon of gasoline equivalent) from the blends when compared to microalgae alone (94.7 - 96.5% depending on the blend of SCG). This work demonstrates that SCG can be easily incorporated with microalgae into a combined processing methodology and can therefore be used effectively during periods of lower availability of microalgae maintaining maximum operating levels of the conversion process equipment year-round. Moreover, co-processing algae with SCG not only leads to increased ethanol titers in the fermentation but also improves the lipid extraction yields.

3.2.3 Introduction

Microalgae have been widely demonstrated to be a highly promising candidate for alternative fuel production (1,2). While the majority of research has focused on lipid based fuels, microalgae also contains substantial protein and carbohydrate fractions (3,5). Recently, we demonstrated that by combining processing stages together, termed Combined Algal Processing (CAP), the cost of biofuel production could be reduced substantially compared to a focus on lipids alone (6). The range of products is not limited to fuel products as a range of other bulk chemical precursors have been demonstrated (6,7). The CAP configuration includes an acid pretreatment to depolymerize the carbohydrates into monosaccharides to be fermented to ethanol. The fermented slurry is then submitted to lipid extraction with hexane as the extracting solvent. The solvent phase is separated from the solids and fed into a distillation column to recover the solvents for reuse in extraction and the lipids for upgrading to renewable diesel blendstock. Because a significant portion of algal biomass remains after the fermentation and extraction, the option of maximizing biofuel yields by carrying out hydrothermal liquefaction (HTL) to produce bio-crude was explored (8).

While microalgae can be grown year round, a key challenge associated with future microalgal biorefineries is the lower productivity and consequently, the lower availability of microalgal biomass during winter, autumn and spring seasons (9-12). This low productivity is due to seasonal temperature and insolation fluctuations throughout the year. Therefore, it is challenging to optimize and scale the downstream equipment. Wendt *et al.* studied the possibility to store microalgae in periods of higher supply (spring and summer) to be used in the lower supply seasons (autumn and winter) (13). However, this would require the conversion processes to run at less than peak summer levels, as well as risking loss of biomass quantity and quality through less than optimal storage, making the overall yearly biofuel production rates lower (14).

In this paper, an alternative solution to this challenge is presented, blending microalgae with spent coffee grounds (SCG) in periods of lower productivity (autumn, winter and early spring). With this approach the biorefinery can run at design specification operating levels throughout the entire year. During periods of higher microalgae supply, the operating levels would be in accordance with this availability, while during the periods of lower supply, SCG would be added to make up and keep the biorefinery running at high operating rates.

The United States Department of Agriculture estimates a yearly consumption of 9.8 million tonnes of coffee beans worldwide (15). Depending on the coffee bean origin and on the brewing process, SCG composition includes carbohydrates (42-55 % w/w), triglycerides (10-24 % w/w), protein (10-18 % w/w), lignin (0-25 % w/w), chlorogenic acids (1-3 % w/w), caffeine (0-0.4 % w/w) and ash (1-2 % w/w) (16-21). The high percentages of carbohydrates and triglycerides suggest that this can be a suitable feedstock to be blended with microalgae in the biorefinery concept. In addition, there is the possibility to obtain this feedstock for little or no costs (the only costs to be considered are the collection and transportation costs) and the presence of similar components in both SCG and lignocellulosic biomass. To this end, we aimed to demonstrate the suitability of SCG as a blending feedstock with microalgae in a modified CAP design (Figure 3-1).

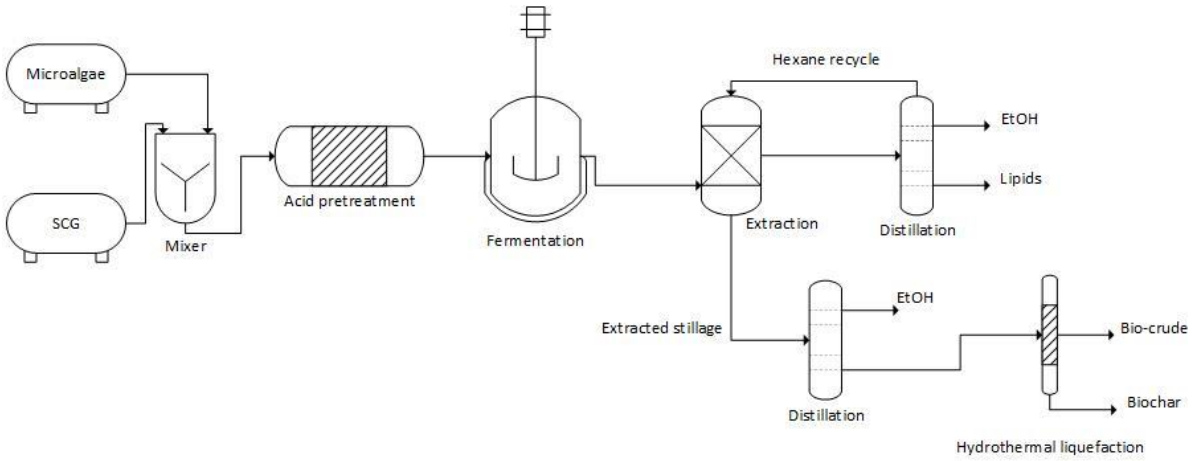


Figure 3-1 – Modified CAP biorefinery process configuration employed in this investigation.

3.2.4 Materials and methods

3.2.4.1 Material acquisition

Scenedesmus acutus 0401 (HCSD) was grown outdoors in flat panel photobioreactors under nitrogen deplete conditions to increase the concentration of lipids and carbohydrates in the biomass. The seed was grown in outdoor reactors using nitrate as the nitrogen source during scale up. A total of 8 reactors, at 660 L/ reactor, were used to produce the biomass. Harvesting was accomplished using Alfa Laval centrifuge (Warren, MI). The harvested biomass was shipped frozen to NREL and stored frozen till needed. Spent coffee grounds, provided by the NREL café, were homogenized by thorough stirring where big agglomerates were broken down to smaller particles. The original composition of these two feedstocks is present in Table 3-1.

Both the algae and spent coffee grounds feedstock samples were prepared as follows: 250 g of sample were weighed into a preweighted cannister; followed by the addition of deionized water to make up a solution of 25% solids (considering the moisture content of both feedstocks – 35.4% for microalgae and 35.9% for spent coffee grounds); sulphuric acid was added to obtain a final solution of 2% H_2SO_4. The blends were then prepared by mixing the two feedstocks in the below mentioned percentages and the same procedure used in the prepared of the pure streams was then followed. The composition of the four slurries prepared are as follows:

- HCSD – *Scenedesmus acutus*
- SCG – spent coffee grounds
- Blend 1 – 40 % HCSD and 60% SCG (w/w) – representing winter
- Blend 2 – 60 % HCSD and 40% SCG (w/w) – representing autumn and early spring

The percentages of microalgae and SCG in blend 1 and 2 representing winter and autumn seasons were based on results obtained from a model previously developed at NREL (22-23).

Table 3-1 – Original feedstock and blends biomolecular composition.

%	*Scenedesmus acutus*	Spent Coffee Grounds
FAME	23.5	16.3
Carbohydrates	38.1	50
Glucose	27.5	9.7
Galactose	1.9	11.2
Mannose	8.7	28.7
Protein	14.7	11
Ash	2.3	2.2
Total	78.6	79.5

3.2.4.2 Acid pretreatment

Pretreatment experiments were carried out in a bath-type ZipperClave® reactor, previously described (6,24,25). 250 g of wet biomass were loaded into the reactor. Water and sulfuric acid were added achieving a 25% (w/w) total solids and 2% (w/w) H_2SO_4 solution (considering the biomass moisture). The reactor was heated up to 155 °C with the aid of steam injection at the bottom of the reactor, increasing the pressure inside the reactor to approximately 5 bar. After 15 minutes the cannister containing the pretreated slurry was removed and cooled in ice water. A set of three replicates for each of the feedstocks studied was conducted to provide enough substrate for fermentation.

A Mettler-Toledo SP precision infrared balance (Columbus, OH) was used to determine the total solid content of biomass at 105 °C. Additional pretreatment determinations were previously described (6).

3.2.4.3 Fermentation

A seed culture of *S. cerevisiae* D5A was grown in YPD at 37 °C in a shake flask at 225 rpm overnight.

The triplicates obtained in the pretreatment experiments were combined and neutralized to an approximate pH of 5. 270 mL of pretreated slurry were added to the fermenters and supplemented with 30 mL of 10x yeast extract-peptone (100 and 200 g/L, respectively) for a total volume of 300 mL. Fermenters were inoculated to an initial OD_{600} of 0.7. Fermentations were run for 48 hours while the fermenters were maintained at pH 5.5 (with 5 M NaOH), 37 °C and stirred at 250 rpm. Samples were taken for HPLC analysis to determine sugar consumption and ethanol production during fermentation.

Control media to replicate the sugars content of either the pretreated algae or SCG contained a base of yeast extract (10 g/L) and peptone (20 g/L). In addition, the algae control media contained approximately 23.9 g/L glucose, 2.9 g/L galactose

and 9.5 g/L mannose, while the spent coffee grounds control media contained 2.7 g/L glucose, 29 g/L galactose and 54 g/L mannose. Periodic fermentation samples were taken for HPLC analysis.

3.2.4.4 Lipid extraction

The fermented slurry was put in contact with hexanes (1:1 ratio, w/w) in Erlenmeyer flasks with overnight agitation on a multi position magnetic stirrer plate (Velp, Bohemia, NY, USA). We have recently learned that ethanol can act as an effective co-solvent with hexanes for higher lipid yields, and so the extraction was performed before ethanol recovery in contrast to an earlier published procedure (6). The samples were then transferred to conical centrifuge tubes and centrifuged for 10 minutes at 2000 g. The organic phase (containing both the hexanes and lipids that migrated from the fermented slurry) was separated and collected in pre-weighted glass vials and subsequently evaporated in a TurboVap concentration workstation (Caliper Life Sciences, East Lyme, CT, USA) at 40 °C. The glass vials were then left overnight in a vacuum oven at 40 °C for further residual solvent evaporation. The glass vials were weighed to determine the total lipids obtained. FAME extraction yields were calculated based on the FAME content of the original feedstock.

3.2.4.5 Hydrothermal liquefaction and analysis

The extracted slurry obtained from the lipid extraction was initially vacuum dried and then freeze dried to remove water, ethanol and any remaining hexanes. 1 g of these solids and 4 g of water were added to the HTL reactors and heated to 300 °C (26). After 30 minutes of reaction time, the reactors were cooled in cold water. The contents of the reactor were then transferred to a separatory funnel. Dichloromethane (DCM) was used to help in the removal of any residual components left in the reactor and transferred to the separatory funnel. This was shaken and left to rest for phase separation. Once the two phases were clearly separated, both were removed and collected in separate pre-weighed vials. The bio-crude phase was submitted to solvent evaporation in a TurboVap concentration workstation at 40 °C followed by overnight evaporation in a vacuum oven at 40 °C. Vials were weighed and the dry bio-crude ash-free yields were determined considering the initial load of solids including ash. Biochar was obtained through filtration of both the organic and aqueous phases when collecting them from the separatory funnel.

3.2.4.6 Analysis

Carbohydrate analysis

Carbohydrate analysis followed the NREL laboratory analytical procedure developed by Van Wychen *et al* (27). This analysis consists of a two-step hydrolysis performed on lyophilized material (original feedstocks and intermediate solids). Approximately 25 mg of each sample was weighed into a pressure tube, followed by the addition of 250 µL of 72% sulfuric acid (Ricca Chemical Company, Arlington, TX) with constant vortexing. Pressure tubes were then placed in a water bath at 30 °C with vortexing 10 to 15 minutes. After 1 hour, samples were diluted with 7 mL of 18.2 mega-ohm water, vortexed and placed in an autoclave for 1 hour at 121 °C. Samples were then cooled down, neutralized to a pH of 6-8 using calcium carbonate and filtered using 0.2 µm nylon filters to HPLC vials.

All liquid fraction samples were analyzed for total and monomeric sugars using the laboratory analytical procedure developed by Sluiter *et al* (28). Monomeric sugar analysis on pretreated liquor was performed by dilution of the sample followed by neutralization to a pH between 608 using calcium carbonate and filtered using 0.2 µm nylon filters into LC vials. Total sugars were determined by one-step hydrolysis where the samples were diluted and 72% sulfuric acid (Ricca Chemical Company, Arlington, TX) was added to make a solution with 4% acid concentration. Samples were autoclaved at 121 °C for 1 hour, let to cool down at room temperature, neutralized with calcium carbonate to pH 6-8 and filtered to an HPLC vial using 0.2 µm nylon filters.

HPLC analysis on carbohydrates on the original feedstocks, total an monomeric sugars was done using a HPLC-RID (Agilent 1100 series, Santa Clara, CA, USA) equipped with a Shodex Sugar SP0810 (300 mm x 8 mm) column (Phenomenex, Torrance, CA, USA) Cation H^+ and Anion CO_3^- de-ashing guard cartridges (Biorad Laboratories, Hercules, CA, USA). Mobile phase was 18.2 mega-ohm water at a flow of 0.6 mL min^{-1}. Column temperature was 85 °C and guard columns were left outside at room temperature.

Monomeric sugars in the fermented samples were analyzed using an HPAEC-PAD system due to the same elution time of mannose and ethanol. The monomeric sugar content of these samples was obtained by dilution and filtration of these samples. The HPAEC-DAD system (Dionex ICS-5000+, Sunnyvale, CA, USA) using a PA-1 column guard. Mobile phase was 14 mM of sodium hydroxide prepared in house from 50% (w/w) sodium hydroxide solution (Fisher Chemical,

Hampton, NH, USA) with a flow rate of 1.0 mL min^{-1}. Both the column and guard were heated up to 35 °C.

Ethanol analysis

Fermentation samples for ethanol analysis were filtered and analyzed for ethanol content using an HPLC-RID (Agilent 1100 series, Santa Clara, CA, USA) equipped with an Aminex HPX-87H (300 mm x 7.88 mm) organic acids column and a Cation H^+ guard column (Biorad Laboratories, Hercules, CA, USA). 0.01 N sulfuric acid was used as mobile phase at a flow of 0.6 mL min^{-1}. Column was heated up to 55 °C. Mobile phase prepared in house using 10 N sulfuric acid (Ricca Chemical Company, Arlington, TX, USA).

FAME analysis

FAME analysis on the raw biomass and extracted FAME was performed following the laboratory analytical procedure developed by Van Wychen *et al*. where 7 to 10 g of sample were weighed in GC vials followed by drying in a vacuum oven at 40 °C for two days (29). 25 µL of internal standard consisting of tridecanoic acid methyl ester, 200 µL of 2 : 1 (v/v) chloroform:methanol and 300 µL of 0.6 M HCl: methanol were added to the samples using gas-tight syringes. Vials were then sealed and vortexed before being placed in a preheated block at 85 °C. After 1 hour, vials were removed from digital dry block and left to cool down at room temperature for no longer than 1 hour. 1 mL of HPLC grade hexane was added to the samples. Samples were vortexed and left undisturbed for 1 hour. A fraction of the upper phase of the samples (FAME in hexane) was removed from the vials, transferred to a new set of GC vials and diluted in hexane depending on the biomass nature. A GC-FID (Agilent 7890B, Santa Clara, CA, USA) system equipped with a DB-Wax capillary column 30 m, 0.25 mm ID and 0.25 µm FT, a 1 µL injection at 10 : 1 split ratio, a constant flow rate of 1 mL min^{-1} of helium, inlet temperature of 250 °C and an oven temperature at 100 °C for 1 minute, 25 °C min^{-1} up to 200 °C, hold for 1 minute, 5 °C min^{-1} up to 250 °C, hold for 7 minutes, FID at 280 °C with 450 mL min^{-1} zero air, 40 mL min^{-1} of hydrogen and 30 mL min^{-1} of helium.

Protein analysis

Percent protein was performed by determining the nitrogen percentage in the samples (slurry, liquor or lyophilized solid material) and then using 4.78 as a conversion factor to obtain final protein percentage (30). For original biomass or intermediate solid samples, approximately 5 to 10 mg (depending if it is original

biomass or intermediate solid, respectively) of lyophilized material was weighted on a small tin foil sheet, which was then folded and pressed into a packet. For liquid and slurry samples, 10 or 20 mg of samples, respectively, was weighted into a small tin foil capsule. Nitrogen analysis was performed in an Elementar Vario El Cube CHN Analyzer (Ronkonkoma, NY, USA). Samples were combusted at a 950 °C in an oxygen rich environment, where the produced gas was run through a GC column and detected *via* a thermal conductivity detector.

Moisture and ash analysis

The laboratory analytical procedure developed by Van Wychen *et al.* was used to determine the moisture and ash content in the samples (31). Approximately 25 mg of biomass was weighed into pre-weighed crucibles, which were placed in an oven at 40°C for two days. Crucibles were removed from the oven, cooled at room temperature and weighed to determine moisture content. Same crucibles were then placed in a muffle furnace increasing the temperature as follows: 12 min at 105°C, followed by an increase to 250°C at 10°C/min, 30 min at 250°C, followed by an increase at 20 °C min^{-1} until 575°C, 180 min at 575°C, then a temperature decrease and held at 105°C. The crucibles were then cooled at room temperature and weighed to determine the ash content.

3.2.4.7 Theoretical conversion yields calculations

Theoretical conversion yields were calculated assuming that all fermentable sugars are being converted to ethanol with a 51% theoretical yield and the fatty acid are converted to renewable diesel with a 78 wt.% theoretical yield (32,33). The HTL bio-oil calculation was made using the Demirbas equation (Eq. 3.1) to determine the energy content of the bio-oil produced (34).

$$HHV\ [MJ/kg] = 33.5(C) + 142.3(H) - 15.4(O) \quad \text{Eq. 3.1}$$

Where C, H and O are the percentages of carbon, hydrogen and oxygen, respectively, in the bio-oils obtained. All the results were converted to MJ equivalent for comparison reasons and then to gasoline equivalents considering 1 gasoline gallon equivalent is 122.48 MJ (35). Conversion from bio-oil to fuel was assumed to be 100% (36). This is then converted to metric units (L/tonne).

3.2.5 Results and discussion

3.2.5.1 Acid pretreatment

The aim of pretreatment is to depolymerize the carbohydrates present in the feedstock into fermentable sugars. The pretreatment of HCSD gave a high level of glucose, up to 26.2 g/L (Figure 3-2), approximately 44% of the available glucose in the algal biomass is therefore released as monomeric glucose at this stage. These yields are calculated based on the sugar concentration before and after pretreatment. The monomeric yield is obtained by the ratio of the monomeric glucose after pretreatment divided by the total (monomeric and oligomeric) glucose in solution before pretreatment. Additionally, lower quantities of galactose and mannose are also released with a substantial proportion being present as oligosaccharides. Alternatively, SCG does not contain substantial levels of glucose, and only 12% of the original glucose present in the SCG is released during pretreatment to monomeric glucose. This suggests that at least some of the glucan present in SCG is in a recalcitrant form, possibly cellulose. It does not appear that the low glucose yields were due to degradation of the glucose to hydroxymethyl furfural (HMF) during pretreatment because HMF levels did not exceed 1.1 g/l in the liquor phase suggesting that the pretreatment severity was not excessively high. However, high levels of galactose and mannose are recovered from the pre-treatment, demonstrating that both algal and SCG hydrolysates would be suitable for further fermentation. A blend of both the HCSD and SCG released approximately the sugar profile that would be expected from the proportion of SCG added. In total the sum of sugars available for fermentation is therefore higher with the blends than the algal biomass alone.

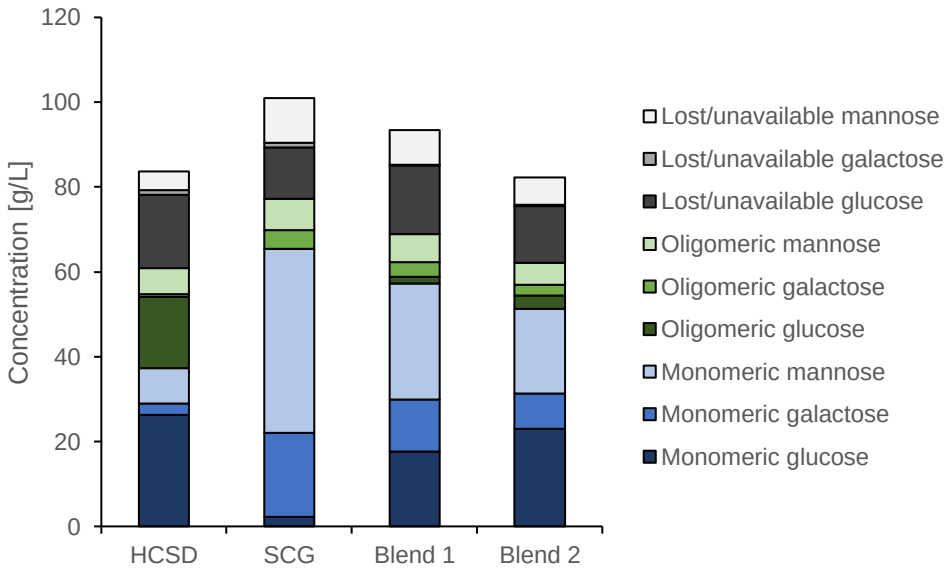
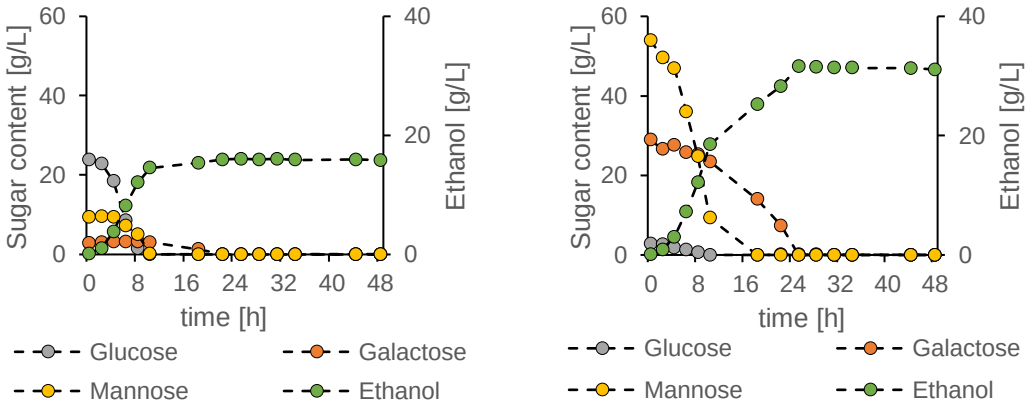


Figure 3-2 – Major fermentable sugars released from the pre-treatment, as monosaccharides (blue), oligosaccharides (green) and sugars either converted to inhibitors or unavailable for fermentation (grey).

3.2.5.2 Fermentation

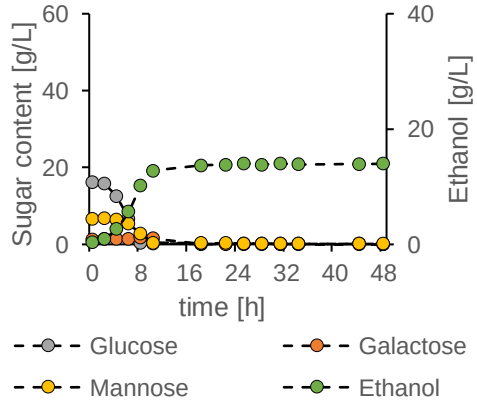
The resulting slurries from pretreatment were pH adjusted and fermented (Figure 3). In order to determine if the slurries contained inhibitory compounds, control fermentations having the same sugar profiles as the HCSD and SCG slurries were also investigated. The monomeric sugars were consumed at approximately the same rate in the controls as compared to the slurries and is suggestive that there are no nutrient limitations or inhibitory compounds in either the HCSD or SCG slurries (Figure 3-3 a-d). *S. cerevisiae* is well suited for these fermentations because it can metabolize all three of the major sugars present in both algae and SCG. All sugars were consumed within 24 hours, with some diauxic behavior observed with glucose being consumed preferentially.

The blends of SCG and HCSD behaved similarly with ethanol concentrations of 20.3 g/L achieved for blend 1 and 18.6 g/L for blend 2 falling between the concentrations obtained for microalgae (14.0 g/L) and SCG (22.7 g/L) (Figure 3-3).

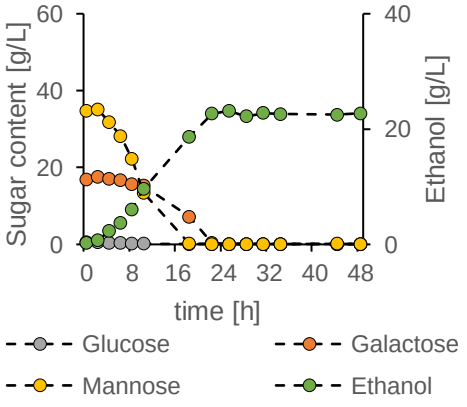


(a) Control HCSD

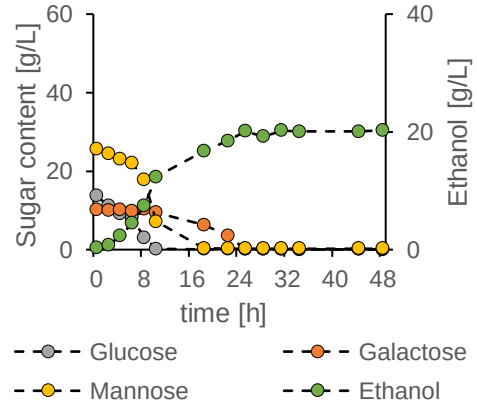
(b) Control Spent coffee grounds



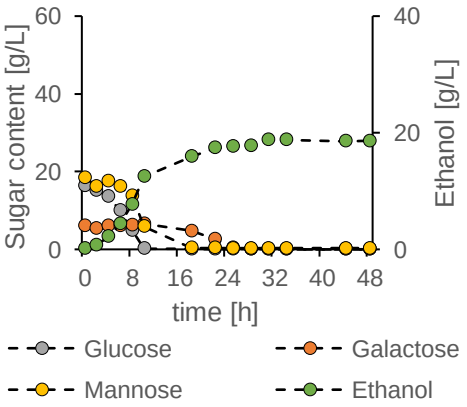
(c) HCSD



(d) Spent Coffee Grounds



(e) Blend 1



(f) Blend 2

Figure 3-3 – Sugar consumption and ethanol production during fermentation of the various feedstocks

The ethanol produced from the fermentation was compared to the theoretical maximum, based on the monomeric sugar content of the pretreated slurry assuming a 51% theoretical ethanol fermentation yield (24,32). The yields ranged from 77-94% (Table 3-2) demonstrating the suitability of fermentation after pretreatment.

Table 3-2 – Ethanol titers and yields in the fermentation for the four pretreated slurries

	Titer [g/L]	Yield (%)
Control HCSD	15.8 ± 0.0	85.6 ± 2.6
Control SCG	31.1 ± 0.0	71.3 ± 4.6
HCSD	14.0 ± 0.2	93.6 ± 0.3
SCG	22.7 ± 0.0	80.3 ± 2.7
Blend 1	20.3 ± 0.2	76.8 ± 2.6
Blend 2	18.6 ± 0.1	86.7 ± 4.9

3.2.5.3 Lipid extraction

After fermentation, the lipids were recovered from the fermented slurry using three successive rounds of hexane extraction (Figure 3-4), with a combined yield of >92% from the algae and blend materials. This demonstrates that lipid is not being consumed or degraded by the yeast during fermentation and the high yield is due to the presence of ethanol produced by fermentation acting as a co-solvent. However, the lipid yields obtained on the fermented SCG alone, were considerably different from the other fermented slurries. In the first extraction the yield was considerably lower than the other samples, and overall only 68% of the original lipid was recovered. This was presumably due to the formation of a double layer observed during the agitation of the SCG fermented slurry with the extraction solvent, while this double layer was not observed for microalgae and both blends. The surface of the SCG might be more hydrophilic and resistant to hexane mass transfer. There are small amounts of surface active compounds (e.g. peptide, protein, polar lipids) in algal biomass and these surfactant might help reduce the surface tension between the hexane and biomass, increased hexane mass transfer for a better extraction (37). On the other hand, stable emulsion caused by surfactant is not favored for phase separation after the extraction, but we noticed that emulsion was not stable after the extraction and could easily be broken by gravimetric settling or a centrifugation. The higher lipid yield in a blend system indicates that lipid yield was improved by the presence of algal biomass. This problem could be solved by increasing the agitation of extraction or adding a fourth extraction step. However, as this was not observed with the blends, it is unlikely to be a problem in the biorefinery system.

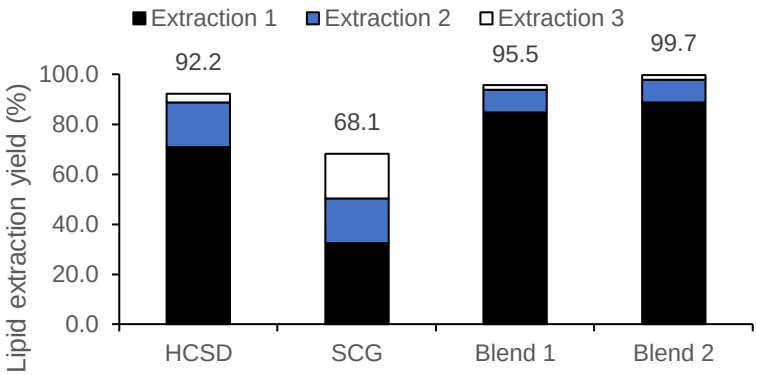


Figure 3-4 – 1st, 2nd and 3rd extraction yields. These yields are calculated based on the amount of lipids obtained in extraction and the lipids in the original feedstock

Applied to a biorefinery scenario and given the increased lipid extraction efficiency of the blends, an increase in total lipids extracted is realized from the blends over algae or SCG alone (Table 3-3).

Table 3-3 – Percentage of lipids extracted and total mass of lipids extracted per tonne of dry biomass extrapolated to a biorefinery scenario

	% lipids extracted	Total lipids extracted [$kg_{lipids}/tonne_{biomass,\ dry}$]
HCSD	92.2	216.9 ± 3.2
SCG	68.1	90.8 ± 1.2
Blend 1	95.5	173.8 ± 3.5
Blend 2	99.7	193.5 ± 0.1

The extracted lipids were converted into fatty acid methyl esters (FAME) to assess the lipid profile (Figure 3-5). The fatty acid extracted from HCSD was predominantly oleic acid, whereas from the SCG, was linoleic and palmitic. This is in keeping with the typical fatty acid profile of both feedstocks (16, 38). The lipid extracted from the blends was a direct mixture of the two profiles.

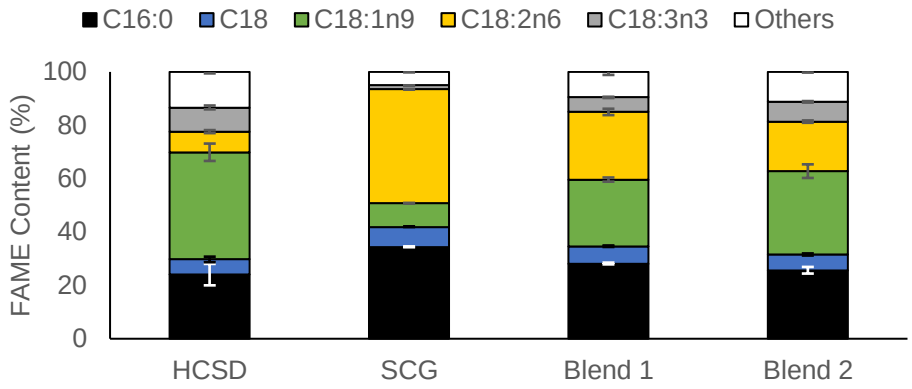


Figure 3-5 – Lipid composition (FAME composition as an average between the three extractions)

The composition of residual solids remaining after fermentation, extraction of the lipids, and drying is given in Table 3-4. This material is rich in unfermented, complex, polymeric carbohydrates and protein. The sum of all the percentages of all the components specified is approximately 70%. Such low mass closure can be explained by the presence of unquantified compounds in algal biomass (*e.g.* nucleic acids, algaenan, moieties from polar lipids, *etc*) and unquantified compounds from SCG (*e.g.* lignin, caffeine and chlorogenic acids). After the consumption of monomeric carbohydrates during fermentation and the extraction of lipids, these unquantified compounds make-up a larger proportion in the residual solids.

Table 3-4 – Residual solids composition

	Carbohydrates (%)	Lipid (%)	Protein (%)	Ash (%)
HCSD	22.3 ± 0.7	1.9 ± 0.2	30.7 ± 0.2	14.4 ± 0.5
SCG	21.3 ± 1.3	5.1 ± 0.8	25.2 ± 0.1	16.7 ± 0.8
Blend 1	23.3 ± 1.5	2.8 ± 0.6	28.3 ± 0.2	14.7 ± 0.6
Blend 2	20.1 ± 0.1	1.9 ± 0.2	29.8 ± 0.3	14.8 ± 0.1

3.2.5.4 Hydrothermal liquefaction

The residual solids still have large amounts of organic carbon which has the potential to be valorized into further useful components (8). To this end they were converted *via* hydrothermal liquefaction. The dried solids were reconstituted with water and subjected to HTL, yielding bio-oil to further improve the biofuel yield of the biorefinery. The bio-crude yields are given in Table 3-5.

Table 3-5 – HTL bio-crude gravimetric yields (dry ash free basis)

	Bio-crude gravimetric yield (%)	Bio-char gravimetric yield (%)
HCSD	24.6 ± 0.32	11.0 ± 2.5
SCG	20.0 ± 6.0	6.4 ± 3.5
Blend 1	17.5 ± 5.1	9.4 ± 3.4
Blend 2	19.7 ± 6.3	13.6 ± 0.9

The yields were reasonably similar, ranging from 18 to 25%, being the highest for microalgae and the lowest for the blend 1, which is the blend with higher percentages of SCG. Unlike the other conversion processes employed, the bio-crude results for the blends do not fall between the results for SCG and microalgae, which correlates with the reduced lipid content in the blends when compared to the residual produced from the SCG (Table 3-5).

3.2.5.5 Biofuel precursor yields in a biorefinery scenario

The CAP scheme, as applied to microalgae and typical seasonal blends with SCG in a biorefinery scenario, produces three biofuel precursors: bioethanol, lipids and bio-crude. To directly compare the overall output of each fractionation and recovery step, the energy content of each fraction was converted into gasoline equivalents (Table 3-6). The HTL bio-crude energy content (Table 3-7) was calculated based on its elemental composition (as described in the methods section). All the values were then converted to the same units (MJ equivalent) and finally to gasoline equivalents in metric units (L per tonne).

Table 3-6 – Fuel yields in the four feedstocks in a potential biorefinery scenario.

Theoretical calculations	HCSD	SCG	Blend 1	Blend 2
Total Carbohydrates (% DW)	38	50	45	43
Ethanol (% DW)[a]	19	25	23	22
Gasoline equivalent (L/Tonne)[b]	162	211	191	182
MJ equivalent	4754	6191	5616	5329
Fatty Acids (FAME) (% DW)	24	16	19	21
Hydrocarbon (% DW)[c]	18	13	15	16
Diesel equivalent (L/Tonne)	216	150	176	189
MJ equivalent	6519	4530	5325	5723
HTL bio-oil (% DW)[d]	5	4	4	4
bio-oil MJ equivalent[e]	1558	1123	1212	1332
Total Gasoline Equivalent (L/Tonne)	437	404	414	422

[a] 51% glucose-to-ethanol theoretical conversion; [b] 65.8% ethanol-to-gasoline conversion; [c] 78% FAME-to-hydrocarbon theoretical conversion; [d] HTL experimental results; [e] based on experimental results and equation 1

Table 3-7 – CHN analysis of bio-crude and respective HHV calculated using Eq. 3.1

	Elemental Analysis				
	C (%)	H (%)	N (%)	O (%)[a]	HHV [MJ]
HCSD	74.1 ± 0.3	8.6 ± 0.1	9.2 ± 0.0	8.1 ± 0.3	35.8
SCG	72.8 ± 0.7	8.2 ± 0.1	9.2 ± 1.0	9.8 ± 0.2	34.6
Blend 1	74.0 ± 0.5	7.9 ± 0.1	9.0 ± 0.2	9.0 ±0.5	34.7
Blend 2	75.0 ± 1.9	8.2 ± 0.4	9.1 ± 0.3	7.8 ± 2.5	35.6

[a] Determined by the difference between the totals and the sum of carbon, hydrogen and nitrogen percentages.

Excitingly, the seasonal inclusion of SCG blended with algae into the CAP process produces similar levels of fuel energy (measured in gasoline equivalents). While HCSD produced the highest gasoline equivalent value (437 L/tonne), the blends with SCG were comparable producing between 414-422 gasoline equivalent L/tonne. This is a reduction of between 3.5 - 5.3% respectively, in the energy produced compared to when HCSD is used. This is mainly due to the higher carbohydrate content, leading to more ethanol which is less energy dense than renewable diesel from lipids. And these numbers could be higher if the pretreatment resulted in higher monomeric sugar yields, because carbohydrates are a better feedstock for fermentation to ethanol than for HTL where they primarily contribute to biochar production (39). Further development of this process to better match the feedstock would be warranted to maximize total biofuel yields and reduce overall production costs. Such results suggest that these blends can be effective to mitigate periods of lower microalgae supply to maintain biofuel precursor production with minimal overall impact.

3.2.6 Conclusion

In this investigation SCG were assessed to evaluate whether they could be used to make up the shortfall in microalgae production in colder seasons of the year (winter and autumn). To this end, blends of *Scenedesmus acutus* and SCG were co-processed in the CAP process, previously demonstrated to have higher economical potential than alternative algal platforms. The aim of the acid pretreatment step was to depolymerize the macromolecules in the feedstock into fermentable sugars. The pretreatment results were satisfactory as the blends yielded higher concentrations of fermentable sugars (glucose, galactose and mannose) than the microalgae feedstock. These sugars were then all consumed in the fermentation leading to higher quantities of ethanol produced (20.3 g/L for blend 1 and 18.6 g/L for blend 2) compared to the 14.0 g/L produced in HCSD. The lipids extracted from the fermented slurries of the blends resulted in higher overall yields, though this represented a slight reduction in the total amount of lipids extracted in the blends (62-68 kg/tonne of wet biomass) compared to the lipids extracted from HCSD alone (76.8 kg/tonne of wet biomass) because SCG had a lower lipid content than algae. Finally, the residual solids left after the lipid extraction were used as feedstock in an HTL process to produce bio-crude. The gravimetric yields obtained in this process for the different feedstocks were relatively similar, ranging from 18 to 25% (AFDW).

To assess the potential of these blends compared to pure microalgae, the energy content of the three fuel products was compared. Although the blends led to a lower total gasoline equivalent than HCSD, the differences registered are relatively small (5.3% for the blend representing winter and 3.5% for the blend representing autumn). This work demonstrates that SCG can be effectively used as a blend in microalgae-based biorefineries using the CAP configuration during periods of lower supply of this feedstock. These results also open up the possibility that SCG alone could serve as a feedstock for a multi-product biorefinery concept similar to that proposed for algal biomass. While it is true that the energy released per tonne of SCG was less than either algae alone or the two algae/SCG blends, yield improvements are to be expected with further process developments. In addition SCG, unlike algal biomass, is already being produced at a volume of 9.8 million tonnes per year and largely relegated to landfills or low value products. This work offers the opportunity to evaluate the commercial potential for large-scale collection of SCG for production of fuels and chemicals.

3.2.7 Acknowledgements

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3.2.8 References

1. Chisti Y. Biodiesel from microalgae. Biotechnol Adv. Elsevier; 2007 May 1;25(3):294–306.

2. Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: A review. Renew Sustain Energy Rev. Pergamon; 2010 Jan 1;14(1):217–32.

3. Volkman JK, Brown MR, Dunstan GA, Jeffrey SW. THE BIOCHEMICAL COMPOSITION OF MARINE MICROALGAE FROM THE CLASS EUSTIGMATOPHYCEAE1. J Phycol. 1993;29(1):69–78.

4. Brown MR. The amino-acid and sugar composition of 16 species of microalgae used in mariculture. J Exp Mar Bio Ecol. Elsevier; 1991 Mar 5;145(1):79–99.

5. Dineshkumar R, R N, Jayasingam P, Sampathkumar P. Cultivation and Chemical Composition of Microalgae Chlorella vulgaris and its Antibacterial Activity against Human Pathogens. J Aquac Mar Biol. 2017;5:19.

6. Dong T, Knoshaug EP, Davis R, Laurens LML, Van Wychen S, Pienkos PT, et al. Combined algal processing: A novel integrated biorefinery process to produce algal biofuels and bioproducts. Algal Res. Elsevier; 2016 Nov 1;19:316–23.

7. Knoshaug EP, Mohagheghi A, Nagle NJ, Stickel JJ, Dong T, Karp EM, et al. Demonstration of parallel algal processing: production of renewable diesel blendstock and a high-value chemical intermediate. Green Chem. The Royal Society of Chemistry; 2018;20(2):457–68.

8. Li Y, Leow S, Dong T, J. Nagle N, P. Knoshaug E, M. L. Laurens L, et al. Demonstration and Evaluation of Hybrid Microalgae Aqueous Conversion Systems for Biofuel Production. ACS Sustain Chem & Eng. American Chemical Society; 2019 Feb 17;7(6):5835–44.

9. Wigmosta MS, Coleman AM, Skaggs RJ, Huesemann MH, Lane LJ. National microalgae biofuel production potential and resource demand. Water Resour Res. 2011;47(3).

10. R. Venteris E, L. Skaggs R, M. Coleman A, S. Wigmosta M. A GIS Cost Model to Assess the Availability of Freshwater, Seawater, and Saline

Groundwater for Algal Biofuel Production in the United States. Environ Sci & Technol. 2013 Apr 5;47(9):4840–9.

11. Coleman AM, Abodeely J, Skaggs R, Moeglein WAM, Newby DT, Venteris ER, et al. An Integrated Assessment of Location-Dependent Scaling for Microalgae Biofuel Production Facilities. Algal Res 579-94.

12. Moody JW, McGinty CM, Quinn JC. Global evaluation of biofuel potential from microalgae. Proc Natl Acad Sci. National Academy of Sciences; 2014;111(23):8691–6.

13. Wendt LM, Kinchin C, Wahlen BD, Davis R, Dempster TA, Gerken H. Assessing the stability and techno-economic implications for wet storage of harvested microalgae to manage seasonal variability. Biotechnol Biofuels. 2019 Apr;12(1):80.

14. Spiller R, Knoshaug EP, Nagle N, Dong T, Milbrandt A, Clippinger J, et al. Upgrading brown grease for the production of biofuel intermediates. Bioresour Technol Reports. Elsevier; 2020 Feb 1;9:100344.

15. United States Department of Agriculture. Coffee: World markets and Trade. 2018.

16. Massaya J, Prates-Pereira A, Mills-Lamptey B, Benjamin J, Chuck CJ. Conceptualization of a spent coffee grounds biorefinery: a review of existing valorisation approaches. Food Bioprod Process. 2019;in press.

17. Cruz R, Cardoso MM, Fernandes L, Oliveira M, Mendes E, Baptista P, et al. Espresso Coffee Residues: A Valuable Source of Unextracted Compounds. J Agric Food Chem. 2012;60(32):7777–84.

18. Ballesteros LF, Teixeira JA, Mussatto SI. Chemical, Functional, and Structural Properties of Spent Coffee Grounds and Coffee Silverskin. Food Bioprocess Technol. 2014 Dec;7(12):3493–503.

19. Karmee SK. A spent coffee grounds based biorefinery for the production of biofuels, biopolymers, antioxidants and biocomposites. Waste Manag. 2017;

20. Somnuk K, Eawlex P, Prateepchaikul G. Optimization of coffee oil extraction from spent coffee grounds using four solvents and prototype-scale extraction using circulation process. Agric Nat Resour. Elsevier; 2017 Jun 1;51(3):181–9.

21. Martinez-Saez N, García AT, Pérez ID, Rebollo-Hernanz M, Mesías M, Morales FJ, et al. Use of spent coffee grounds as food ingredient in bakery products. Food Chem. Elsevier; 2017 Feb 1;216:114–22.

22. Davis RE, Markham JN, Kinchin CM, Canter C, Han J, Li Q, et al. 2017 Algae Harmonization Study: Evaluating the Potential for Future Algal Biofuel Costs, Sustainability, and Resource Assessment from Harmonized Modeling.

23. Quinn JC, Davis R. The potentials and challenges of algae based biofuels: a review of the techno-economic, life cycle, and resource assessment modeling. Bioresour Technol. England; 2015 May;184:444–52.

24. Laurens LML, Nagle N, Davis R, Sweeney N, Van Wychen S, Lowell A, et al. Acid-catalyzed algal biomass pretreatment for integrated lipid and carbohydrate-based biofuels production. Green Chem [Internet]. The Royal Society of Chemistry; 2015;17(2):1145–58.

25. Weiss ND, Nagle NJ, Tucker MP, Elander RT. High xylose yields from dilute acid pretreatment of corn stover under process-relevant conditions. Appl Biochem Biotechnol. United States; 2009 May;155(1–3):418–28.

26. Dong T, Xiong W, Yu J, Pienkos PT. Co-production of fully renewable medium chain α-olefins and bio-oil via hydrothermal liquefaction of biomass containing polyhydroxyalkanoic acid. RSC Adv. The Royal Society of Chemistry; 2018;8(60):34380–7.

27. Van Wychen S, Laurens LML. Determination of total carbohydrates in algal biomass: laboratory analytical procedure (LAP). 2016.

28. Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, et al. Determination of Structural Carbohydrates and Lignin in Biomass—NREL/TP-510-42618. Lab Anal Proced. 2008.

29. Van Wychen S, Ramirez K, Laurens LML. Determination of Total Lipids as Fatty Acid Methyl Esters (FAME) by in situ Transesterification: Laboratory Analytical Procedure (LAP).

30. Lourenço SO, Barbarino E, Lavin PL, Marquez UML, Aidar E. Distribution of intracellular nitrogen in marine microalgae: Calculation of new nitrogen-to-protein conversion factors. Eur. J. Phycol.; 2004; 39(1): 17-32.

31. Van Wychen S, Laurens LML. Determination of Total Solids and Ash in Algal Biomass: Laboratory Analytical Procedure (LAP). 2016.

32. Balat M, Balat H. Recent trends in global production and utilization of bio-ethanol fuel. Appl Energy. 2009 Nov;86(11):2273–82.

33. Davis R, Fishman D, Frank ED, Wigmosta MS, Aden A, Coleman AM, et al. Renewable Diesel from Algal Lipids: An Integrated Baseline for Cost, Emissions, and Resource Potential from a Harmonized Model.

34. DEMIRBAS A, GULLU D, ÇAGLAR A, AKDENIZ F. Estimation of Calorific Values of Fuels from Lignocellulosics. Energy Sources. Taylor & Francis; 1997;19(8):765–70.

35. Alternative Fuel Data Center USD of E. Alternative fuel comparison. 2014.

36. Tzanetis KF, Posada JA, Ramirez A. Analysis of biomass hydrothermal liquefaction and biocrude-oil upgrading for renewable jet fuel production: The impact of reaction conditions on production costs and GHG emissions performance. Renew Energy. Pergamon; 2017 Dec 1;113:1388–98.

37. Dong T, Knoshaug EP, Pienkos PT, Laurens LML. Lipid recovery from wet oleaginous microbial biomass for biofuel production: A critical review. Appl Energy. Elsevier; 2016 Sep 1;177:879–95.

38. Damiani MC, Popovich CA, Constenla D, Martínez AM, Doria E, Longoni P, et al. Triacylglycerol content, productivity and fatty acid profile in Scenedesmus acutus PVUW12. J Appl Phycol. 2014 Jun;26(3):1423–30.

39. Leow S, Witter JR, Vardon DR, Sharma BK, Guest JS, Strathmann TJ. Prediction of microalgae hydrothermal liquefaction products from feedstock biochemical composition. Green Chem. The Royal Society of Chemistry; 2015;17(6):3584–99.

Chapter 4

An integrated biorefinery to produce 5-hydroxymethylfurfural and alternative fuel precursors from macroalgae and spent coffee grounds

4.1 Context

Macroalgae represent another source of biomass that does not compete with land for food production. However, unlike microalgae, that can be grown in arid lands, macroalgae are cultivated in both saltwater and freshwater systems. This can take place both through aquacultures, the specific farming of targeted macroalgal strains or through wild harvesting in bloom areas. Therefore, macroalgae presents an opportunity to countries with extensive coastlines, or that simply do not have the land area for extended microalgal cultivation.

Like other biomass sources, macroalgae require appropriate conditions for their growth. While some species prefer warmer water temperatures, others prefer colder waters, and extensive biomass production is observed even in climates not suited to microalgal growth. The availability of nutrients and solar exposition are variables that lead to macroalgae with different biomolecular compositions. In addition, it is rare to be able to source just one species from any system. Therefore, an integrated biorefinery using macroalgae as its main feedstock, must always take into account the type of macroalgae and its biomolecular composition.

To this end, the present study aimed to convert two species of macroalgae, *Ulva lactuca* and *Chorda filum*, collected in the South West of the United Kingdom, in an integrated biorefinery design to produce 5-hydroxymethylfurfural (HMF). These two macroalgae belong to two different families, *Chlorophyta* (green algae) and *Heterokontophyta* or *Ochrophyta* (brown algae), respectively. The composition of these macroalgae may be different from other samples of the same species collected elsewhere, which might lead to different results in the present biorefinery design.

Similarly to the microalgae presented in chapter 3, the cultivation and processing of macroalgae also presents a seasonality problem. To tackle this issue, macroalgae was blended with spent coffee grounds (SCG). Two blends were studied for each of the macroalgae species used. The blend with lower percentage of macroalga represents the season corresponding to the lower production, while the blend with higher percentage of macroalga represents intermediate seasons. The blends were tested in parallel with the pure feedstocks, both macroalgae and SCG.

Additionally to the production of HMF, this approach also investigated the co-production of a biocrude oil and bio-char, using hydrothermal liquefaction.

This chapter is submitted in an alternative format in line with Appendix 6A of the “Specifications for Higher Degree Theses and Portfolios” as required by the University of Bath.

All the experimental work in this chapter was undertaken by the author apart from the lipid analysis that was performed by Dr. Tim Woodman, a co-author on this paper.

The paper is to be submitted to the RSC journal Sustainable Energy and Fuels.

4.2 Sustainable Energy and Fuels paper

An integrated biorefinery to produce 5-(hydroxymethyl)furfural and alternative fuel precursors from macroalgae and spent coffee grounds

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4.2.1 Key words

Integrated biorefinery

Macroalgae

Macroalgae seasonality

Spent Coffee Grounds

Biofuels

4.2.2 Abstract

5-hydroxymethylfurfural (HMF) is a promising platform chemical produced from the dehydration of C_6 sugars, that is a precursor for a range of renewable fuels and polymers. In this study, an integrated macroalgal biorefinery was designed to produce an array of products including HMF, hydrothermal liquefaction (HTL) biocrude and a biochar. In this process two different species of macroalgae, *Ulva lactuca* and *Chorda filum*, were investigated and co-processed with spent coffee grounds to assess if such blends could be effectively used, with the spent coffee grounds mitigating for lower macroalgae availability throughout the year. *U. lactuca* and the spent coffee ground blends were effectively used in a biorefinery design for the production of HMF. Interestingly, blends yielded higher amounts of HMF (35-47 g per kg of dry biomass processed) than the separate components alone. This is presumably due to the elevated amount of C_6 sugars being available from the macroalgae, coupled with the presence of lipids from the coffee grounds. The lipids likely form a separate organic layer in the dehydration reaction, into which the HMF migrates after being formed in the aqueous fraction, halting further dehydration reactions to levulinic acid. The HTL on the resultant solids from dehydration yielded a relatively similar amount of biocrude (68-78 g per kg of dry biomass) compared to spent coffee grounds (SCG) (90 g per kg of dry biomass). However, the *C. filum* biorefinery yielded far lower biocrude and HMF, presumably due to the lower lipid and C_6 sugar content in this feedstock. Overall, an HMF biorefinery from macroalgae is plausible, with spent coffee grounds being a highly suitable material to make up for seasonal availability. However, the large difference in yields from macroalgal species demonstrates the importance of high lipid content, alongside higher C_6 sugar composition, in the macroalgal feedstock.

4.2.3 Introduction

Macroalgae is a promising feedstock for the next generation of biorefineries as it does not compete with food crops, has a higher rate of carbon dioxide fixation than land crops (1), has no freshwater requirement and is simple to process (2). In addition, its cultivation can help to alleviate the eutrophication in seas and oceans and aid in carbon capture and sequestration (3). There are over 30.1 million tonnes currently cultivated worldwide (4,5), predominantly in China and India. The UK has one of the most extensive coastlines in Europe (approximately 12,500 km) and the ideal water temperature for seaweed production. These conditions and the underdeveloped market present a large opportunity for further development (6). There are a wide range of seaweed species growing around the UK, that inevitably, have different properties and components depending on the type, habitat, cultivation method and harvest time (7,8). However, the predominant feature across almost all species are the elevated carbohydrate levels (65-75%) (9).

Several authors have demonstrated that macroalgae can be used in the hydrothermal liquefaction process (10). Raikova et al presented a comprehensive study on a broad range of macroalgae species from the UK and the ideal hydrothermal liquefaction conditions to convert these into bio-crude and nutrient partition into the aqueous phase (11). In a further study the authors also combined plastics present in the ocean with the macroalgae demonstrating that this led to a higher biocrude heating value (12).

However, the use of macroalgae in an HTL-based industry presents two key issues. The first is the relatively low production of crude from the majority of species when compared to microalgae (13). This is primarily because of the elevated carbohydrates levels (relative to the more proteinaceous microalgae), that predominantly breakdown into insoluble biochar, a lower quality fuel than the biocrude (14–16).

Indeed, the majority of research on macroalgal valorisation has focussed on the conversion of the carbohydrate fraction, mainly through fermentation to ethanol (17) and alternative cellular products (18). However, the saccharides must first be extracted and processed into fermentable sugars, there are a high number of competing pathways for carbon in any fermentation and the breadth of sugars that any one organism would need to metabolise make these routes challenging. A simpler, more targeted approach, is the acid catalysed breakdown of the macroalgal saccharides to produce 5-hydroxymethyl furfural (HMF). HMF is a highly promising chemical building block, with a forecasted market of 61 million USD by 2024 (19,20). Its production from carbohydrates and the potential to be converted into high value biofuels make this molecule an important

intermediate between the carbohydrate and petroleum-based industry (21). HMF has the potential to be converted into dimethylfuran (DMF), a biofuel with high energy density; and 2,5-furandicarboxylic acid (FDCA) a building block used in the production of polyethylene 2,5-furandicarboxylate (PEF), an emerging biobased polymer proposed to replace polyethylene terephthalate (PET) (22,23). The production of HMF from macroalgae has been demonstrated including from isolated agar (24) and the red macroalga *Kappaphycus alvarezeii* (25).

Another key issue is the seasonal growth of macroalgae, which does not lend itself to an effective all year supply, thereby limiting the size or scope of a potential biorefinery (26–29). Recent studies have shown that blending with alternative biomass sources can be used to even out supply, and produce products all year round. For example, Jin et al showed that co-liquefaction of microalgae and macroalgae was possible, and even increased the bio-crude energy heating value compared to when the feedstocks were processed separately (30). Similarly, we recently demonstrated that microalgal seasonality can be effectively addressed by blending this feedstock with spent coffee grounds (SCG) in periods where the microalgae production is lower during the colder parts of the year (31). In this biorefinery set-up the saccharides were extracted and fermented before the resulting stillage was processed through HTL. Spent coffee grounds are a promising material for bioprocessing, which are available all year round, relatively stable to store, with the worldwide production of SCG being approximately 10 million tonnes in 2019 (32). The composition of SCG varies but, similarly to macroalgae, they contain high carbohydrates (42-55 w/w %) with a similar C6 composition (33). We recently demonstrated the suitability of producing HMF from spent coffee grounds in an integrated biorefinery design using an organosolv fractionation to isolate cellulose (34).

In this investigation therefore, we aimed to combine these approaches and address two fundamental issues impeding the development of macroalgal biorefineries. To this end, an integrated approach to increase the atom efficiency and produce both HMF and HTL products was demonstrated, co-processing the biomass with SCG to even out seasonality issues and allow steady production all year round.

4.2.4 Methods

4.2.4.1 Materials

Spent coffee grounds were acquired from a local café at the University of Bath. A sample was weighed and placed in the oven at 60 °C. After two days the sample was weighed, and moisture content determined.

Ulva lactuca and *Chorda filum* were sampled from Broadsands Beach, Paignton, UK. *Ulva lactuca* and *Chorda filum* were sampled on the 30/07/2019 and 05/08/2019, respectively. Both macroalgae were washed in freshwater, and freeze dried prior to storage.

In addition to the 'pure' feedstocks of *U. lactuca, C. filum* and spent coffee grounds, two blends were prepared for each macroalgae. The blend with 40% macroalga and 60% SCG simulates a season when there is less production of macroalgae (possibly winter – depending on the strain) and the blend with 60% macroalgae and 40% SCG simulate an intermediate season between maximum and minimum macroalgae production (possibly spring or autumn). Therefore, the seven feedstocks studied are as follows:

- pure *Ulva lactuca* – UL
- Blend 1 – 60% UL + 40% SCG – $UL_{0.6}$ + $SCG_{0.4}$
- Blend 2 – 40% UL + 60% SCG – $UL_{0.4}$ + $SCG_{0.6}$
- pure spent coffee grounds - SCG
- Blend 3 – 40% CF + 60% SCG – $CF_{0.4}$ + $SCG_{0.6}$
- Blend 4 – 60% CF + 40% SCG – $CF_{0.6}$ + $SCG_{0.4}$
- pure *Chorda filum* – CF

4.2.4.2 Acid dehydration

20 g (dry weight) of feedstock were added to a 300 mL Parr reactor (Parr Company Moline, IL, USA, 4560 mini reactors), followed by the addition of a mixture of 2% sulphuric acid (provided by Sigma-Aldrich) in deionised water to perform a total of 100 g. The reactor was weighed before the reaction. Agitation was initiated and the reactor heated to 155 °C, at which point the temperature was held for 15 minutes. The reactor was then cooled down with the help of an in-built cooling tube system supplied with cooling liquid at -4 °C and an ice bath in contact with the outside walls of the reactor. On average, cooling from 155 °C to 30 °C took around 20 minutes. The reactor and its contents were weighed to determine gas losses.

4.2.4.3 HMF extraction

The contents of the reactor were poured into a filter paper in a funnel, which was on top of a separatory funnel. This filtration step was performed as it improves the accuracy of extraction yields – the presence of solids in solution prevents a good distinction between the organic and aqueous phases. The filtrate flows into a 500 mL separatory funnel, whereupon 100 mL of a mixture of dicholoromethane (DCM) and 2-butanol (50:50 w/w %) were added to the filtrate. The separatory funnel was closed, shaken and left to rest until the two phases were clearly separated. Organic phase (containing the extraction solvent and HMF) and aqueous fraction were collected in separate measuring cylinders for volume measure and an aliquot of each was collected for sugar, furfural and HMF quantification in HPLC analysis.

The recovery ratio of HMF in the extraction is calculated as follows:

$$R_{HMF} = \frac{C_{HMF,org} \times V_{org}}{C_{HMF,org} \times V_{org} + C_{HMF,aq} \times V_{aq}} \qquad \text{Eq. 4.1}$$

Where $C_{HMF,org}$ and $C_{HMF,aq}$ are the concentration of HMF in the organic and aqueous fraction, respectively, in g/L. V_{org} and V_{aq} are the volume of the organic and aqueous fraction, respectively.

4.2.4.4 Second acid dehydration

The aqueous phase collected from the HMF extraction was combined with the solids obtained from filtration upstream from HMF extraction. This slurry was then added to the same reactor used in the first acid dehydration and the same reaction procedure was used (temperature, agitation, heating ramp, reaction time and cooling).

4.2.4.5 HMF second extraction

The initial procedure performed in the first HMF extraction was followed with the only difference that the solids were dried overnight in an oven at 40 °C to reduce moisture content to residual quantities and to improve processing via hydrothermal liquefaction. The solids could have been fed to HTL as they were, if the moisture content was known, however this approach would imply a reduction in the amount of added water to HTL based on the moisture present in the solids.

4.2.4.6 Hydrothermal liquefaction (HTL)

HTL reactions were performed in a 50 mL stainless steel batch reactor, equipped with a pressure gauge, pressure relief valve and a needle valve. 3 g of dried solids were

weighed, loaded and mixed with 15 g of deionised water into an HTL reactor. The reactor was tightly sealed (due to high pressures) and placed in a furnace at 800 °C. Temperature was closely monitored using a thermocouple until it reached 350 °C (approximately between 150 and 180 bar). At this temperature the reactor was removed from the furnace and left to cool at and to room temperature. Gas phase was measured and collected from the needle valve (using water displacement technique). Reactor was opened and its contents were poured onto a pre-weighed filter paper for separation of the aqueous phase from the insoluble biocrude and biochar. The filtrate (aqueous phase) was poured through the funnel into a pre-weighed vial. The vial and the filtrate obtained were weighed for total aqueous fraction weight determination. An aliquot of the aqueous fraction was oven-dried at 60 °C to determine the aqueous fraction residue yield. The reactor and the filtered solids were then thoroughly washed (using the same filter paper) with chloroform into a pre-weighed round bottom flask until the filtrate was clean. A rotary evaporator was used for chloroform removal at 40 °C. Once the solvent was evaporated, the flask was weighed and the biocrude fraction gravimetric yield was obtained. Solids were dried in an oven at 60 °C. Filtered solids were weighed for biochar gravimetric yield determination.

4.2.4.7 Analysis

Carbohydrates and levulinic acid analysis

Aqueous and organic fractions obtained after the first and second extractions were filtered and analysed for carbohydrate content using an high performance liquid chromatography (HPLC), from Agilent Technologies, equipped with an Aminex HPX-87H organic acids column (300 mm x 7.88 mm, Bio-Rad Laboratories) and a refractive index detector (RID) was used to quantify the carbohydrates in this study. 5mM H_2SO_4 solution was used as mobile phase at a flow rate of 0.6 mL/min. Column was heated up to 65 °C. Mobile phase was prepared using sulphuric acid provided from Sigma-Aldrich.

HMF and furfural analysis

An HPLC (Agilent Technologies) equipped with a diode-array detector (DAD) and an Aminex HPX-87H column (300 mm x 7.88 mm, Bio-Rad Laboratories) was used in the HMF and furfural analysis on the aqueous and organic samples obtained after both extractions. The mobile phase (5mM H_2SO_4 solution prepared in house using sulphuric acid provided from Sigma-Aldrich) was flowing at 0.6 mL/min. Column was heated up to 65 °C. DAD signal set at 280 nm.

Lipid analysis

Lipid composition was determined using ^{1}H nuclear magnetic resonance (NMR). The use of NMR techniques to study olive oil is well established and reasonable methods exist based on integration of the signals for distinct components of the triglyceride (Figure 4-1) (35,36). The composition can be calculated by combining the various signal intensities, such as the glyceryl proton at 5.25 ppm, the unsaturated protons between 5.3 and 5.45 ppm, and the bis-allylic protons (linoleic and linolenic) at 2.76 and 2.80 ppm. Notably several different approaches to these calculations have been reported, but all are in good agreement with data obtained via GC methods. The method used herein was derived from that of Seijas *et al*, however rather than use a 750 MHz spectrometer to reduce the overlap of the bis-allylic protons, a simple homo-decoupling step was used to reduce the overlapped triplets to singlets, allowing for a direct determination of the ratio of linoleic and linolenic components (Figure 4-2) (37–39).

A further complication is the high presence of free fatty acids in the samples under study. This was corrected for by comparison of the glyceryl peak and that for the β-CH2 signal. For a triglyceride this is 1:6. Where free fatty acids are present the ratio will increase and the amounts can be determined. The overall composition of the alkyl chains can be determined, as the chemical shifts of the relevant protons virtually identical for free fatty acid and triglyceride.

Some of the samples contained an unexpected peak in the bis-allylic region, notably CF samples. After homo-decoupling, there are three peaks present between 2.75 and 2.85 ppm, rather than the expected two (for linoleic and linolenic, Figure 4-3). Although not fully confirmed, it is likely that this peak arises from stearidonic acid (Figure 4-4), based on the chemical shift.

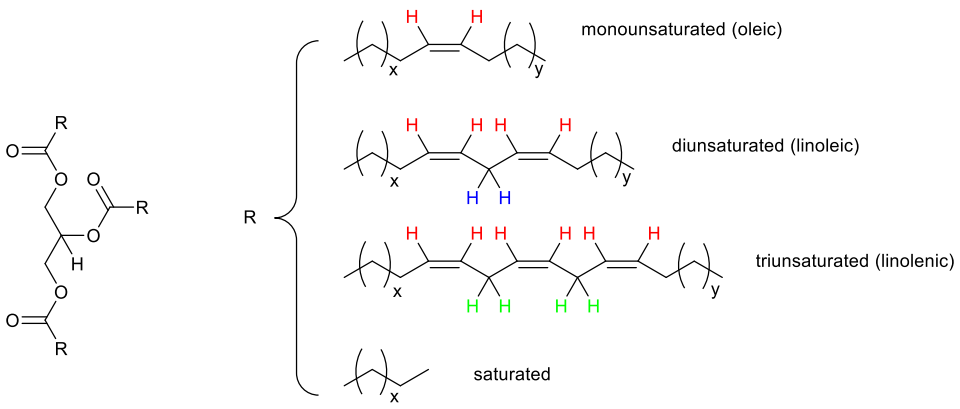
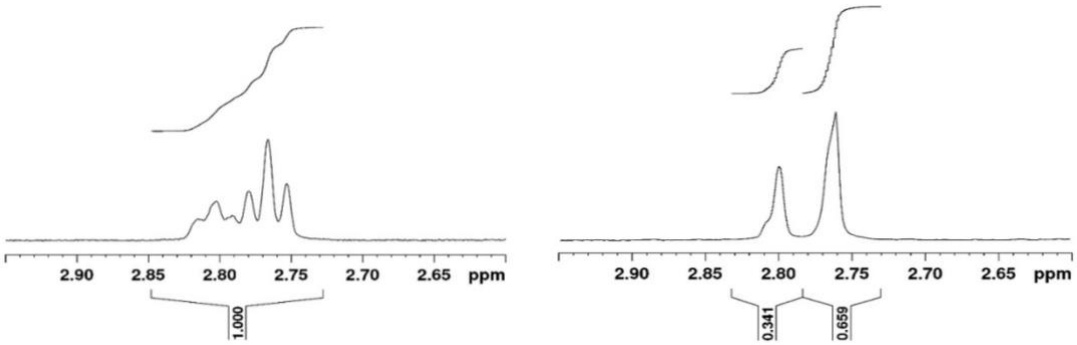


Figure 4-1 – Structure of triglycerides, where the alkyl chain may be mono-, di-, tri-unsaturated or saturated. ^{1}H NMR signals for the tertiary hydrogen of the glycerine head unit, the alkene protons and the vinyl protons can be used to calculate the relative composition of the fatty acids.



(a) (b)

Figure 4-2 – (a) Bis-allylic protons of linoleic (right) and linolenic (left) as overlapping broad triplets. (b) Fully resolved singlets following homo-decoupling of the unsaturated protons, allowing for direct integration.

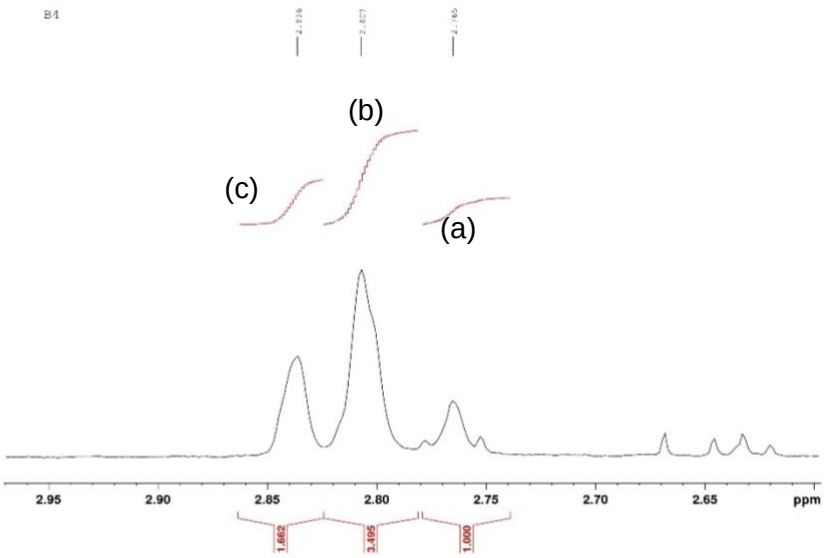


Figure 4-3 – Bis-allylic protons for C. filum. Peak labelled (a) for linoleic acid, (b) is for linolenic, (c) an undefined peak, which may be stearidonic acid.

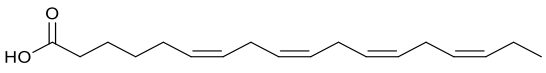


Figure 4-4 – Stearidonic acid

4.2.5 Results and discussion

The biorefinery approach was designed to produce HMF from the C_6 sugar fraction in an acid dehydration. By using H_2SO_4, both the depolymerisation of polysaccharides into oligo and monosaccharides and the subsequent dehydration into HMF should be possible. This process was followed by an extraction of the produced HMF, where the solvent (dichloromethane: 2-butanol (50:50 w/w %) was recycled. The extracted stillage from this extraction was then fed into a second acid dehydration to produce further HMF from the unused carbohydrates of the first reaction. The reactor effluent was subject to a second HMF extraction with the solvent being recycled. The resulting waste stream from the entire process was then submitted to hydrothermal liquefaction to produce biocrude and biochar (Figure 4-5).

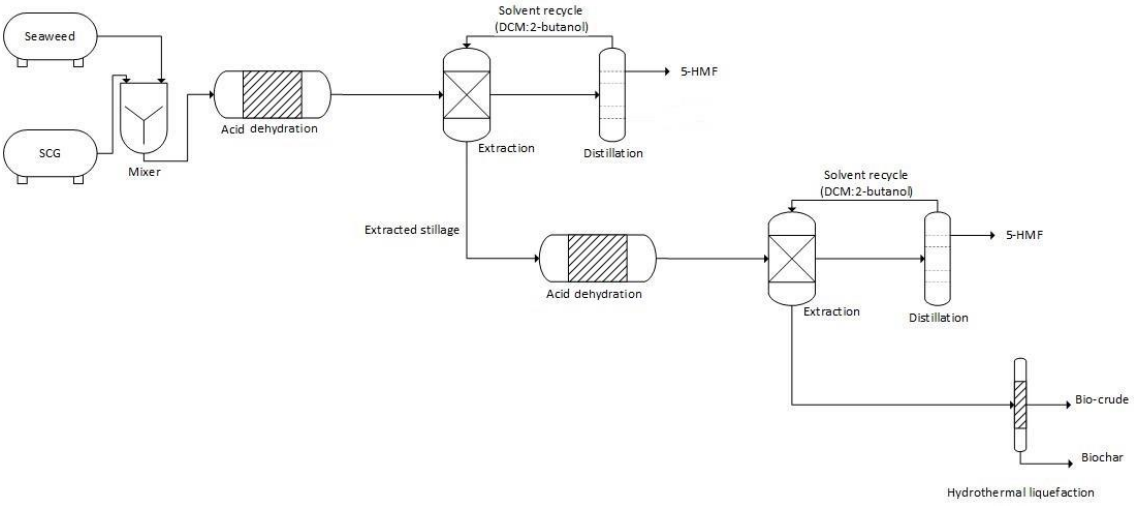


Figure 4-5 – Proposed process configuration of biorefinery for HMF production

SCG and *U. lactuca* had a total carbohydrate content of 51.5 % and 34.7 %, respectively. All of the found carbohydrates in these feedstocks were C_6 sugars (Table 4-1). Such results suggest that these feedstocks can have a high potential for their conversion into HMF.

Table 4-1 – Total carbohydrate content in the pure feedstocks in the literature (40,41)

	SCG	*U. Lactuca*	*C. filum*
Total carbohydrates (%)	51.5 ± 1.7	34.7 ± 1.0	29.2 ± 3.3

4.2.5.1 Acid dehydration for HMF production

The initial sulphuric acid treatment depolymerised a portion of the saccharide feedstock, and some HMF production was observed. The SCG produced the most, 2.69 g/L produced, a yield of 2.6% from the saccharide portion of the coffee. *U. lactuca* produced 1.76 g/L (2.5%) whereas *C. filum* produced 0.24 g/L (0.4%) (Figure 1-1 (a)). Assuming that the concentration of total carbohydrates is proportional to the percentages of each feedstock in the blend, the yields of HMF were calculated. Yields of 7.7% and 7.3% were achieved for $UL_{0.6}$ + $SCG_{0.4}$ and $UL_{0.4}$ + $SCG_{0.6}$, respectively, while 6.0% and 3.5% were obtained for the blends of SCG with CF ($CF_{0.4}$ + $SCG_{0.6}$ and $CF_{0.6}$ + $SCG_{0.4}$, respectively) (Table 4-2).

Interestingly, the blends of Ulva and SCG produced far more HMF than either of the raw materials when processed separately. This was also observed for blends of SCG and CF. This is potentially due to the macroalgal species having more glucose, which is more readily released than in SCG, while the far higher content of lipids in SCG forms an organic layer (9,42–44). It has been previously observed, that a biphasic system can increase stability and yields by partitioning HMF as it is formed away from the aqueous phase (45). This notion is supported by a reduction of approximately 50% on the HMF produced from the blend when defatted SCG were used in the same series of experiments (Figure 4-6 (c)).

All the systems examined have low C_5 sugar content, and accordingly lower furfural production. The reactor effluent was analysed for carbohydrates (Figure 4-6 (b)), and still showed a relatively high content in carbohydrates (approximately 14-23 g/L depending on feedstock). The analysis also showed that mannose is the monosaccharide most abundant in the SCG-rich feedstocks, while higher concentrations of glucose are observed in UL-rich feedstocks and mannitol/arabinose and fucose in CF. Levulinic acid was also present in these slurries, mostly in the feedstocks containing SCG, suggesting that there is significant levels of HMF to dehydrate with the systems containing lipids, with far less observed for the two macroalgae species on their own. The presence of such high concentrations of monosaccharides in this stream indicates that these can be further processed in a second acid dehydration in the production of HMF.

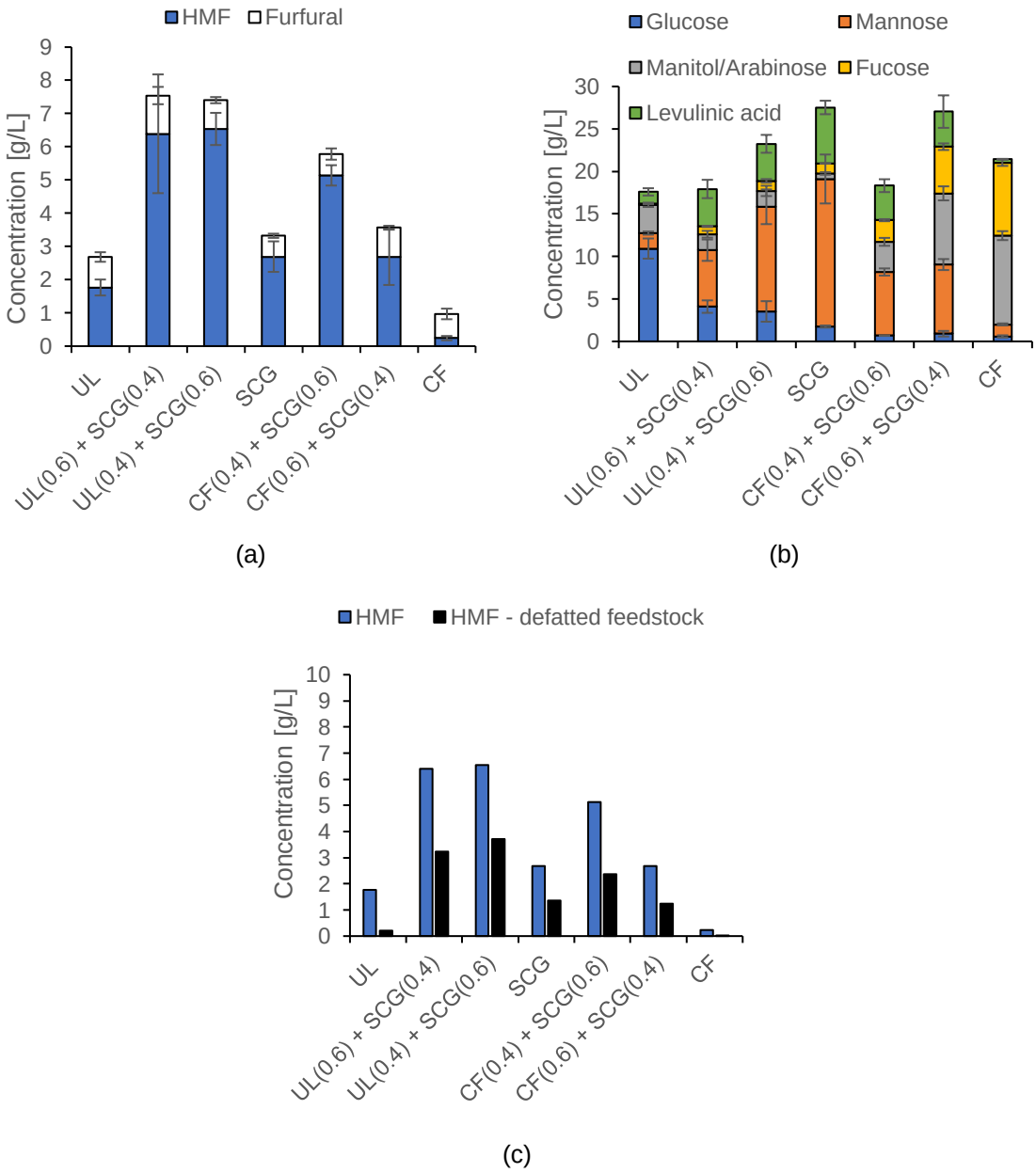


Figure 4-6 – (a) HMF and furfural production in 1st reaction; (b) monosaccharides concentration after 1st reaction; (c) comparison of the HMF produced when using the raw feedstocks and when using a defatted feedstock

Table 4-2 – HMF recovery yields of the various feedstocks and blends. Values obtained considering the theoretical carbohydrate content from literature and the HMF concentration obtained on the first acid dehydration

	UL	$UL_{0.6}+SCG_{0.4}$	$UL_{0.4}+SCG_{0.6}$	SCG	$CF_{0.4}+SCG_{0.6}$	$CF_{0.6}+SCG_{0.4}$	CF
HMF yield (%)	2.5	7.7	7.3	2.6	6.0	3.5	0.4

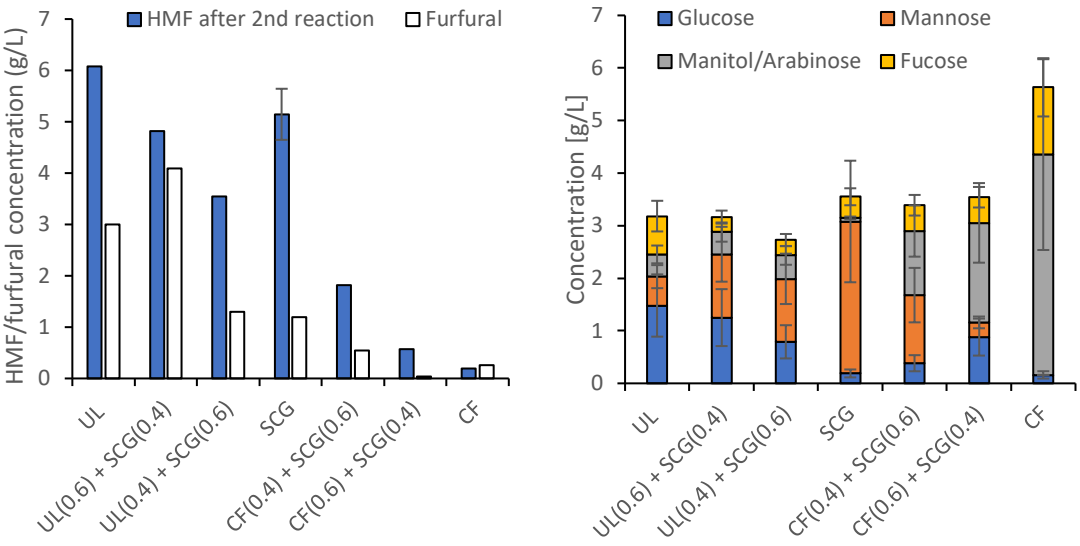
Prior to the second dehydration the organic fraction, containing HMF, was removed. This was to prevent further dehydration of the HMF product while also allowing for more HMF to be migrate to the organic layer formed by the lipids. The extraction system used was a mixture of DCM and 2-butanol in a ratio of 1:1, which showed relatively high recovery ratios for the HMF – approximately 80 to 90% of the HMF was recovered into the extraction solvent, while the rest remained in the aqueous fraction (Table 4-3).

Table 4-3 – HMF recovery yield after first extraction. These percentages were calculated considering the total HMF produced after the acid dehydration and the HMF recovered in the organic fraction.

	UL	$UL_{0.6}+SCG_{0.4}$	$UL_{0.4}+SCG_{0.6}$	SCG	$CF_{0.4}+SCG_{0.6}$	$CF_{0.6}+SCG_{0.4}$	CF
HMF recovery (%)	86.7	80.5	83.1	83.1	82.3	90.4	78.3

4.2.5.2 Subsequent acid dehydration to HMF

A second acid dehydration was undertaken on the combined solid and aqueous phase once the HMF had been removed. This led to higher production of HMF for the aqueous phase produced from the pure feedstocks of UL (6.1 g/L) and SCG (5.1 g/L), though the HMF from the CF (0.2 g/L) and blends of the macroalgae with SCG were greatly reduced (Figure 4-7 (a)). This is presumably that there is a large pool of glucose and mannose that can be dehydrated in the SCG and UL and far less in the CF. In addition, the lack of lipids in CF does not allow for a large production of HMF, similarly to what happened in the first dehydration (Figure 4-7 (a)). However, the HMF produced in the blends of CF is considerably lower than what was achieved in the first dehydration. This might be due to the low amounts of C6 sugars left in solution after the first dehydration (Figure 4-6 (b)). And when compared to the sugars present in solution after the second dehydration (Figure 4-7 (b)), sugars such as mannitol and fucose were barely consumed.



(a) (b)

Figure 4-7 – (a) HMF and furfural concentration in the slurry after the second acid dehydration; (b) sugar concentration in the slurry after the second acid dehydration.

The sugar analysis demonstrates that most of the glucose and mannose are consumed in the dehydration into HMF, while fucose and mannitol are somewhat more stable.

4.2.5.3 Lipid analysis

The streams of HMF (both after first and second dehydrations) and the stream of stillage after the second dehydration were analysed for lipid content. Lipids were only found in the stream of stillage after the second dehydration. This demonstrated that no lipids were extracted with the HMF, which confirms the lipids form an organic phase in both acid dehydrations. This double layer allows for the HMF to move into the organic phase, once produced in the aqueous phase. Even though this was not part of this study, the presence of lipids in the stillage after the second dehydration would also potentially allow for a lipid extraction prior to the HTL reaction. The extraction of the lipids as a product in a separate stream could add further value to this biorefinery approach. Lipids have been widely used in the literature towards the production of biodiesel by a transesterification (46–49). The analysis on the lipids present in the stillage after the second dehydration show a high content of saturated fatty acids as well as linoleic and linolenic acids (Table 4-4).

Table 4-4 – Type of fatty acids found in the lipids in the extracted stillage after the second acid dehydration. MUFA stands for mono-unsaturated fatty acids. Analysis and quantification of fatty acids in UL and CF was difficult due to the low content of fatty acids in these samples. However, in addition to the fatty acids shown below, CF revealed the presence of an unusual component, most probably stearidonic acid.

	Saturated FA	MUFA	Linoleic acid	Linolenic acid
UL	-	-	-	-
$UL_{0.6}$ + $SCG_{0.4}$	58.60	4.10	30.92	6.38
$UL_{0.4}$ + $SCG_{0.6}$	54.35	16.40	27.27	1.98
SCG	56.45	10.18	32.35	1.02
$CF_{0.4}$ + $SCG_{0.6}$	63.67	0.10	30.75	5.48
$CF_{0.6}$ + $SCG_{0.4}$	66.57	0.00	27.67	10.87
CF	-	-	-	-

4.2.5.4 HTL

On extraction of the HMF, the resulting solid was converted into further products through hydrothermal liquefaction. The *U. lactuca*, SCG and respective blends yielded relatively similar bio-crude and biochar yields, with a slight increase in biocrude with higher percentage of SCG in the blends. This is due to the higher content of lipids in SCG, which is converted to biocrude in this process. These results prove that blends of *U. lactuca* with SCG can be used in an effective HTL process without substantial reduction in main product yields (biocrude and biochar).

The same trend was observed with SCG and *C. filum*, where a higher percentage of SCG in the blend associated with a higher biocrude yield. This is presumably due to the higher lipid content in SCG (converted into biocrude in HTL). On the other hand, the biochar yield increases with higher percentages of *C. filum*. Such result is attributed to the fact that most of the sugars were not used in any of the dehydration processes and were broken down into biochar in HTL. In contrast to *U. lactuca, C. filum* blends with SCG led to considerable differences in the biocrude and biochar yields. Therefore, if SCG were to be blended with *C. filum* in a *C. filum* based biorefinery, the processes downstream to HTL would need to be prepared to handle different volumes of biocrude and biochar, depending on the season, thus on the percentages of the blends used.

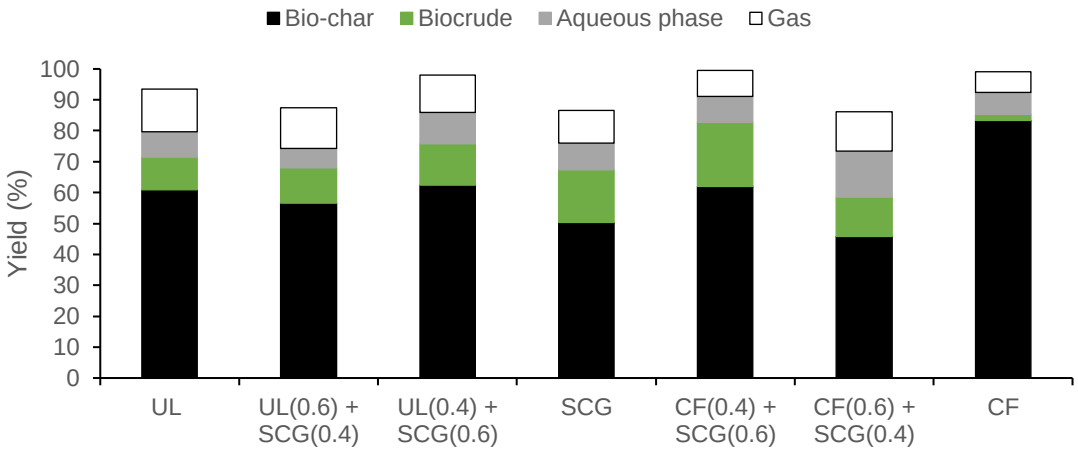


Figure 4-8 – Mass balance of the extracted stillage used in HTL reaction. Gravimetric yields calculated based on the dry weight of the extracted stillage fed into the reaction. Gas phase calculated assuming a 100% content in CO_2, while the aqueous gravimetric yield was calculated considering the solids in this phase.

4.2.5.5 Overall yields

The overall product yields were compared across the different blends and feedstocks (Table 4-5). The total HMF presented is the sum of the HMF extracted after the first and second dehydrations. Yields were extrapolated to 1 tonne of dry biomass fed into the process. The biocrude and biochar amounts were calculated based on the amount of solids obtained after 2nd dehydration and on the HTL yields presented in Figure 4-8. These were also extrapolated to 1 tonne of dry biomass supplied to the entire system.

A higher amount of HMF was produced from $UL_{0.6}$ + $SCG_{0.4}$ (46.6 kg/tonne$_{biomass}$) and $UL_{0.4}$ + $SCG_{0.6}$ (35.3 kg/tonne$_{biomass}$). These are the blends intended to replace *U. lactuca* in periods of intermediate and lower supply of this seaweed. However, when processed separately, *U. lactuca* and SCG only produce 31.2 and 30.2 kg/tonne$_{biomass}$, respectively, demonstrating that the blending of *U. lactuca* and SCG creates improved conditions for HMF production. A higher level of bio-crude was produced for the SCG (89.5 kg/tonne$_{biomass}$) presumably due to the higher content of lipids in this feedstock, though a similar conversion was observed for all biomass blends. However, the higher the percentages of *U. lactuca* in the blends, the higher the production of biochar. This is mainly due to the higher content of ash in the macroalgae. Overall, both $UL_{0.6}$ + $SCG_{0.4}$ and $UL_{0.4}$ + $SCG_{0.6}$ demonstrate to be a good replacement of *U. lactuca* in periods of lower seaweed supply.

Unlike the *U. lactuca* blends, when taking both extractions into account, the highest level of HMF was produced from the SCG and the lowest from the *C. filum.* The blends of

SCG and *C. filum* were proportional to this. Such low production of HMF from the macroalgae is due to the low content of C_6 sugars and lipids in this feedstock. This result has a high impact on the HTL results as the carbohydrates that were not dehydrated into HMF were then broken down into char, resulting in a high production of char of 757.2 kg/tonne$_{biomass}$. In addition to this, the low content of lipids in this feedstock led to a lower biocrude production (14.6 kg/tonne$_{biomass}$) when compared to SCG (89.5 kg/tonne$_{biomass}$).

Table 4-5 – Overall yield of the fuel products and precursors including total produced HMF, lipid production, biocrude and biochar from the proposed biorefinery, all values are given as kg/tonne and calculated on a dry ash free basis.

		UL	$UL_{0.6}$ + $SCG_{0.4}$	$UL_{0.4}$ + $SCG_{0.6}$	SCG	$CF_{0.4}$ + $SCG_{0.6}$	$CF_{0.6}$ + $SCG_{0.4}$	CF
Total HMF extracted [kg/tonne$_{biomass}$]		31.2	46.6	35.3	30.2	28.3	13.1	3.7
HTL	Biocrude [kg/tonne$_{biomass}$]	83.5	78.2	67.6	89.5	109.7	77.5	14.6
	Biochar [kg/tonne$_{biomass}$]	489.8	390.2	314.2	264.2	355.5	230.5	757.2
Mass all fuel products		60.5%	51.5%	41.7%	38.4%	49.4%	32.1%	77.5%
Mass all fuel produts (DAF)		72.0%	57.3%	45.0%	39.0%	51.9%[a]	34.4%[a]	86.1%[a]

[a] ash content obtained from literature as there was not enough macroalgae to run ash content analysis (50)

4.2.6 Conclusion

In this study a biorefinery producing 5-(hydroxymethyl)furfural (HMF), biocrude and biochar from macroalgae and spent coffee grounds blends was developed. *U. lactuca* was found to be a suitable species, containing elevated C_6 sugars that could be converted into HMF. Interestingly, the addition of spent coffee grounds increased the production substantially, in comparison to the macroalgae or coffee grounds alone. This was presumably due to the formation of a lipid layer in the aqueous phase that reduced the decomposition of HMF to levulinic acid. The stillage from the reaction was also further converted into fuel products through HTL, yielding 46.6 g kg^{-1} HMF, 78.2 g kg^{-1} biocrude, 390 g kg^{-1} biochar in the optimised system. The same system with *C. filum* was less productive, presumably due to lower lipid and C_6 sugars in the macroalgae. This work demonstrates an integrate HMF biorefinery is possible using *U. lactuca,* and that the addition of spent coffee grounds not only would allow all year round production, but could increase the yield of specific target products. Indeed, blending with spent coffee grounds has potential to improve process versatility making biomasses considered unsuitable alone (such as *Chorda filum*) into viable feedstocks.

4.2.7 References

1. Tabassum MR, Xia A, Murphy JD. Potential of seaweed as a feedstock for renewable gaseous fuel production in Ireland. Renew Sustain Energy Rev. 2017;68:136–46.
2. Jones ES, Raikova S, Ebrahim S, Parsons S, Allen MJ, Chuck CJ. Saltwater based fractionation and valorisation of macroalgae. J Chem Technol Biotechnol. 2020.
3. Balina K, Romagnoli F, Blumberga D. Seaweed biorefinery concept for sustainable use of marine resources. Energy Procedia. 2017;128:504–11.
4. The state of world fisheries and aquaculture. 2012.
5. The state of world fisheries and aquaculture. 2018.
6. Capuzzo E, McKie T. Seaweed in the UK and abroad - status, products, limitations, gaps and Cefas role. 2016.
7. Murphy J, Drosg B, Allen E, Jerney J, Xia A, Herrmann C. A perspective on algal biogas. IEA Bioenergy. 2015;5-35.
8. Bunker F, Brodie JA, Maggs CA, Bunker AR. Seaweeds of Britain and Ireland. 2nd ed. 2017;312.
9. Beacham TA, Cole IS, DeDross LS, Raikova S, Chuck CJ, Macdonald J, Herrera L, Ali T, Airs RL, Landels A, Allen MJ. Analysis of seaweeds from South West England as a biorefinery feedstock. Appl Sci. 2019;9(20):4456.
10. Raikova S, Allen MJ, Chuck CJ. Hydrothermal liquefaction of macroalgae for the production of renewable biofuels. Biofuels, Bioprod Biorefining. 2019;13(6):1483–504.
11. Raikova S, Le CD, Beacham TA, Jenkins RW, Allen MJ, Chuck CJ. Towards a marine biorefinery through the hydrothermal liquefaction of macroalgae native to the United Kingdom. Biomass and Bioenergy. 2017;107:244–53.
12. Raikova S, Knowles TDJ, Allen MJ, Chuck CJ. Co-liquefaction of Macroalgae with Common Marine Plastic Pollutants. ACS Sustain Chem Eng. 2019;7(7):6769–81.
13. Piccini M, Raikova S, Allen MJ, Chuck CJ. A synergistic use of microalgae and macroalgae for heavy metal bioremediation and bioenergy production through hydrothermal liquefaction. Sustain energy fuels. 2019;3(1):292–301.
14. Biller P, Ross AB. Potential yields and properties of oil from the hydrothermal

liquefaction of microalgae with different biochemical content. Bioresour Technol. 2011;102(1):215–25.

15. Yang W, Li X, Li Z, Tong C, Feng L. Understanding low-lipid algae hydrothermal liquefaction characteristics and pathways through hydrothermal liquefaction of algal major components: crude polysaccharides, crude proteins and their binary mixtures. Bioresour Technol. 2015;196:99-108.

16. Yu G, Zhang Y, Schideman L, Funk TL, Wang Z. Hydrothermal liquefaction of low lipid content microalgae into bio-crude oil. Transactions of the ASABE. 2011;54(1):239-246.

17. Ghadiryanfar M, Rosentrater KA, Keyhani A, Omid M. A review of macroalgae production, with potential applications in biofuels and bioenergy. Renew Sustain Energy Rev. 2016;54:473–81.

18. Abeln F, Fan J, Budarin VL, Briers H, Parsons S, Allen MJ, Henk DA, Clark J, Chuck CJ. Lipid production through the single-step microwave hydrolysis of macroalgae using the oleaginous yeast Metschnikowia pulcherrima. Algal Res. 2019;38:101411.

19. Zhao H, Holladay JE, Brown H, Zhang ZC. Metal Chlorides in Ionic Liquid Solvents Convert Sugars to 5-Hydroxymethylfurfural. Science. 2007;316(5831):1597–600.

20. National Science Foundation. Breaking the chemical and engineering barriers to lignocellulosic biofuels: Next generation hydrocarbon biorefineries. 2008.

21. Bicker M, Hirth J, Vogel H. Dehydration of fructose to 5-hydroxymethylfurfural in sub- and supercritical acetone. Green Chem. 2003;5(2):280–4.

22. Roman-Leshkov Y, J Barrett C, Y Liu Z, A Dumesic J. Production of Dimethylfuran for Liquid Fuels from Biomass-Derived Carbohydrates. Nature. 2007;447:982–5.

23. Kucherov FA, Gordeev EG, Kashin AS, Ananikov VP. Three-Dimensional Printing with Biomass-Derived PEF for Carbon-Neutral Manufacturing. Angew Chemie Int Ed. 2017;56(50):15931–5.

24. Kim B, Jeong J, Shin S, Lee D, Kim S, Yoon H-J, Cho JK. Facile Single-Step Conversion of Macroalgal Polymeric Carbohydrates into Biofuels. ChemSusChem. 2010;3(11):1273–5.

25. Lee S-B, Kim S-K, Hong Y-K, Jeong G-T. Optimization of the production of platform chemicals and sugars from the red macroalga, Kappaphycus alvarezii. Algal Res. 2016;13:303–10.

26. Malea P, Chatziapostolou A, Kevrekidis T. Trace element seasonality in marine macroalgae of different functional-form groups. Mar Environ Res. 2015;103:18–26.

27. Lefèvre C, Bellwood D. Seasonality and dynamics in coral reef macroalgae: Variation in condition and susceptibility to herbivory. Mar Biol. 2010;157:955–65.

28. Fulton CJ, Depczynski M, Holmes TH, Noble MM, Radford B, Wernberg T, Wilson SK. Sea temperature shapes seasonal fluctuations in seaweed biomass within the Ningaloo coral reef ecosystem. Limnol Oceanogr. 2014;59(1):156–66.

29. Ferrari R, Gonzalez-Rivero M, Ortiz JC, Mumby PJ. Interaction of herbivory and seasonality on the dynamics of Caribbean macroalgae. Coral Reefs. 2012;31(3):683–92.

30. Jin B, Duan P, Xu Y, Wang F, Fan Y. Co-liquefaction of micro- and macroalgae in subcritical water. Bioresour Technol. 2013;149:103–10.

31. Prates Pereira A, Dong T, Knoshaug EP, Nagle N, Spiller R, Panczak B, Chuck CJ, Pienkos PT. An alternative biorefinery approach to address microalgal seasonality: blending with spent coffee grounds. Sustain Energy Fuels. 2020

32. Coffee: World Markets and Trade. 2019.

33. Massaya J, Prates-Pereira A, Mills-Lamptey B, Benjamin J, Chuck CJ. Conceptualization of a spent coffee grounds biorefinery: a review of existing valorisation approaches. Food Bioprod Process. 2019;118:149-166.

34. Prates Pereira A, Woodman TJ., Brahmbhatt P, Chuck CJ. The optimised production of 5-(hydroxymethyl)furfural and related products from spent coffee grounds. Appl Sci. 2019;9:3369.

35. Sacchi R, Addeo F, Paolillo L. 1H and 13C NMR of virgin olive oil. An overview. Magn Reson Chem. 1997;35(13):133-145.

36. Vlahov G. Application of NMR to the study of olive oils. Prog Nucl Magn Reson Spectrosc. 1999;4(35):341–57.

37. Feás X, Estevinho LM, Salinero C, Vela P, Sainz MJ, Vázquez-Tato MP, Seijas JA. Triacylglyceride, antioxidant and antimicrobial features of virgin Camellia oleifera, C. reticulata and C. sasanqua Oils. Molecules. 2013;18(4):4573–87.

38. Salinero C, Sanchez X, Mansilla J, Seijas J, Vázquez-Tato M, Vela P, Sainz MJ. H-1-Nuclear Magnetic Resonance Analysis of the Triacylglyceride Composition of Cold-Pressed Oil from Camellia japonica. Molecules. 2012;17(6):6716–27.

39. Vela P, Vázquez-Tato M, Seijas J, Sainz MJ, Mansilla J, Feaz X, Salinero C. NMR analysis of the triglyceride composition of cold pressed oil from Camellia Reticulata and Camellia Japonica. 2011;754.

40. Olsson J, Toth GB, Albers E. Biochemical composition of red, green and brown seaweeds on the Swedish west coast. J Appl Phycol. 2020.

41. Ballesteros LF, Teixeira JA, Mussatto SI. Chemical, Functional, and Structural Properties of Spent Coffee Grounds and Coffee Silverskin. Food Bioprocess Technol. 2014;7(12):3493–503.

42. Massaya J, Prates-Pereira A, Mills-Lamptey B, Benjamin J, Chuck CJ. Conceptualization of a spent coffee grounds biorefinery: a review of existing valorisation approaches. Food Bioprod Process. 2019;118:149-66.

43. Yaich H, Garna H, Besbes S, Paquot M, Blecker C, Attia H. Chemical composition and functional properties of Ulva lactuca seaweed collected in Tunisia. Food Chem. 2011;128(4):895–901.

44. Khotimchenko SV. Fatty acids of brown algae from the Russian far east. Phytochemistry. 1998;49(8):2363–9.

45. Gallo JMR, Alonso DM, Mellmer MA, Dumesic JA. Production and upgrading of 5-hydroxymethylfurfural using heterogeneous catalysts and biomass-derived solvents. Green Chem. 2013;15(1):85–90.

46. Musa IA. The effects of alcohol to oil molar ratios and the type of alcohol on biodiesel production using transesterification process. Egypt J Pet. 2016;25(1):21–31.

47. Hariram V, John JG, Seralathan S. Spectrometric analysis of algal biodiesel as a fuel derived through base-catalysed transesterification. Int J Ambient Energy. 2019;40(2):195–202.

48. Tobar M, Núñez GA. Supercritical transesterification of microalgae triglycerides for biodiesel production: Effect of alcohol type and co-solvent. J Supercrit Fluids. 2018;137:50–6.

49. Dong T, Gao D, Miao C, Yu X, Degan C, Garcia-Pérez M, et al. Two-step microalgal biodiesel production using acidic catalyst generated from pyrolysis-derived bio-char. Energy Convers Manag. 2015;105:1389–96.

50. Ross AB, Jones JM, Kubacki ML, Bridgeman T. Classification of macroalgae as fuel and its thermochemical behaviour. Bioresour Technol. 2008;99(14):6494–

504.

Chapter 5

Conclusions and future work

5.1 Conclusions

The aim of this thesis was to demonstrate that spent coffee grounds (SCG) could be effectively used as a feedstock in an integrated biorefinery towards the production of biofuels and value chemicals. While SCG was shown to be suitable for the production of HMF, the best use of the material was found not as the sole feedstock in a biorefinery, but blended with other sources of biomass and integrated in a biorefinery context to mitigate the lower availability of the primary feedstock during certain seasons.

SCG are a rich source of carbohydrates, protein, lignin and lipids as it has been widely demonstrated across the literature. Such composition allows for a wide range of products to be obtained. An organosolv fractionation on SCG was effectively used, yielding three separate streams: a cellulose-enriched fraction, a hemicellulose-enriched fraction and a lignin-enriched fraction. These streams were then used in separate applications.

Lignin-enriched fraction was characterised and assessed as a potential fuel in the biorefinery to recover energy. When analysed for its content, this stream demonstrated high content in depolymerised lignin and fatty acids. Lignin has the potential to be used in applications such as binders and adhesives, additives, emulsifiers, dispersants, and other polymers and chemicals while the main application of lipids across the literature is its conversion to biodiesel through transesterification.

The hemicellulose-enriched fraction showed a relatively high concentration of depolymerised sugars such as galactose and mannose. Such composition has the potential to be used as a substrate in the growth of a *Metschnikowia pulcherrima* yeast. However, this was only possible when using a relatively simple solvent system in the fractionation process – only made of methyl isobutyl ketone (MIBK) and water. When solvent systems using MIBK, water and ethanol or acetone were used, the growth of this yeast was not possible. This is presumably due to the inhibitory behaviour of ethanol and acetone in the aqueous phase towards the microorganisms activity. Therefore, a separation of these components is suggested so that this fraction can be used as a growth media for fermentation.

Finally, the cellulose-enriched fraction was used in the production of 5-hydroxymethylfurfural (HMF). However, the direct conversion of this fraction to HMF demonstrated low yields. Therefore, an enzymatic hydrolysis of this cellulose into glucose was necessary to improve these yields. An enzymatic isomerisation of this glucose into fructose helped to further improve the HMF yields. HMF is considered a valuable renewable building block in modern biorefineries. This is because of its potential to be further upgraded in downstream industries to dimethylfuran (DMF) and 2,5-

furandicarboxylic acid (FDCA). The higher energy density of DMF when compared to bioethanol, and comparable to the one in gasoline, makes DMF a potential replacement for gasoline in the transport sector. FDCA is a monomer used in the production of polyethylene furanoate (PEF) which is a potential replacement for polyethylene terephthalate with lower greenhouse gas emissions. The first research chapter has therefore demonstrated that SCG can be used in the production of HMF and a solid heating fuel in an integrated biorefinery.

Having demonstrated that SCG can be effectively used as a pure feedstock in an integrated biorefinery towards the production of biofuel precursors, SCG were also assessed as a potential blending agent with other biomass sources in integrated biorefinery designs. The second research chapter investigated the blending of SCG with the microalgae *Scenedesmus acutus* in a biorefinery designed to produce bioethanol, lipids, biocrude and biochar. Such an approach intended to demonstrate that the blending of this microalgae with SCG can tackle the low productivity and therefore availability of microalgae for biorefineries in colder seasons, namely autumn and winter in the USA, where the research took place. Two different blends were studied, one representing the lower availability of microalgae during winter, and other simulating the intermediate supply of this feedstock during seasons such as autumn and early spring. The blends were processed separately and compared with the pure feedstocks which were processed in parallel.

In this biorefinery configuration, the feedstock was initially submitted to an acid hydrolysis to depolymerise the biomass macromolecules, namely the depolymerisation of carbohydrates into fermentable sugars, though this would have also released amino acids suitable as a growth media. These sugars were then used in a fermentation with a strain of *Saccharomyces cerevisiae* to produce bioethanol. The fermented broth was then submitted to a combined extraction and distillation with hexane to extract the lipids. The produced bioethanol present in solution helped in the lipid extraction process. In fact, its presence demonstrated increased lipid extraction yields compared to when this process was performed without the presence of bioethanol. This bioethanol was both collected in this distillation and in a subsequent distillation of the resulting solids for further bioethanol recovery. Finally, the resulting extracted stillage was further processed in a hydrothermal liquefaction to yield biocrude and a biochar as the main products.

This study demonstrated that the studied blends can efficiently mitigate the lower availability of microalgae during autumn and winter seasons by maintaining the operating levels of the biorefinery. Additionally, the blends of microalgae with SCG have also demonstrated higher production of bioethanol when compared to the bioethanol obtained

when processing the microalgae as a pure feedstock. If an approach like this is to be used, the biorefinery operating units need to be prepared for the variable quantities of products obtained depending on the type of feedstock/blends used.

Microalgae has a relatively straightforward production that can be carried out on non-arable lands and therefore do not compete with food crops for land. Additionally, microalgae fast growth when compared to terrestrial biomass crops represents another advantage towards microalgae cultivation. Nonetheless, a reasonable large area of land is still required to install the microalgae production farms. At a global scale, there are vast areas of non-arable land that can be used to install such facilities. However, such areas are not equally distributed across the different countries. A solution to this problem is the production of macroalgae in countries with an extensive coastline. This can be done both in freshwater and saltwater, depending on the macroalgae species. The natural growth of wild species of macroalgae in the various aquatic ecosystems, also allows for their harvest and use. Additionally, the growth of macroalgae is a relatively cheap process as these ecosystems provide all the requirements this type of biomass require, namely water, sunlight and nutrients. These features have contributed to the increased interest on the use of macroalgae as feedstock in biorefineries to produce biofuels.

The use of macroalgae in a biorefinery presents the same problem as the one previously discussed on microalgae-based biorefineries, the feedstock seasonality. To this end, the third research chapter assessed the use of blends of macroalgae with spent coffee grounds. Two macroalgae species from two different families were used, *Ulva lactuca* from the family of *Chlorophyta* (green algae) and *Chorda filum* from the family of *Heterokontophyta* or *Ochrophyta* (brown algae). For each of these macroalgae, two blends were studied, one simulating the season with lower availability of this feedstock and other simulating intermediate seasons. Pure feedstocks, both macroalgae and spent coffee grounds, were also processed in the biorefinery configuration to be used as controls.

This integrated biorefinery was designed for the production of HMF and other value products such as lipids, biocrude and biochar. The feedstock was initially processed in an acid dehydration. This conversion unit is used both as a pretreatment to depolymerise the macromolecular biomass structures, and as a dehydration process to convert the depolymerised carbohydrate units into HMF. This product is considerably unstable and requires an organic layer to where it can migrate upon its production. This is provided by the lipids present in the feedstock. This was corroborated by a series of experiments where a lipid extraction was performed on the feedstock previous to its processing in the

acid dehydration, which led to considerably lower amounts of HMF produced. After the acid dehydration, an extraction of the HMF was performed from the reactor effluent. This extraction would allow further production of HMF in a second acid dehydration and its migration to the lipid layer. A second extraction of the produced HMF was performed. The resulting solid stream obtained after the extraction was further valorised in a hydrothermal liquefaction to produce biocrude and biochar.

This study demonstrated that SCG can be blended with macroalgae in this biorefinery design to produce HMF. In fact, the blends of SCG and the two strains of macroalgae yielded higher amounts of HMF than when the macroalgae were processed separately. This is probably due to the higher amount of lipids in the SCG when compared to both strains of macroalgae. The blends of *U. lactuca* yielded much higher concentrations of HMF than the blends of *C. filum*. This occurs presumably due to higher concentrations of C6 sugars in *U. lactuca*, while *C. filum* presents higher concentrations of C5 sugars.

The work described in this thesis has demonstrated that a municipal waste such as SCG can be effectively used in integrated biorefineries towards the production of higher value products. Also, SCG biomolecular composition allows for them to be co-processed with other biomass sources in various biorefinery designs. This intends to tackle the seasonality problem associated to certain types of biomass such as micro and macroalgae.

5.2 Future work

The work presented above has demonstrated that SCG, commonly seen as a low value waste, is a suitable feedstock for an integrated biorefinery

In the first research chapter HMF was produced from a cellulose-enriched fraction obtained through an organosolv fractionation. Such fraction was then submitted to two enzymatic treatments to yield fructose, which demonstrated to be a better substrate towards the production of HMF when compared to both cellulose and glucose. Even though the production of HMF has been validated from this feedstock, an optimisation of the conditions in the various reactions performed can improve the yields obtained in each reaction and therefore the quantities of HMF produced.

The hemicellulose-enriched fraction obtained in the organosolv fractionation was a combination of the liquid phase obtained in the fractionation with two washes of the solids. One of the washes was done with the same solvent system used in the fractionation while the other wash was performed with water. Instead of combining the liquid phase with the two washes, these could be collected separately. Such approach will probably yield a more concentrated liquid phase in carbohydrates which could then be further employed in the production of high-value products. Considering that this fraction is mainly rich in C_6 sugars such as mannose and galactose, a possible application of this fraction could be in the production of HMF, increasing the overall yield of the biorefinery

Further improvements in this fraction could include the removal of the inhibitors such as ethanol and acetone. This would allow for the use of this fraction as a substrate in the growth of yeast and/or microalgae. On one hand, the growth of certain yeast species such as *Saccharomyces cerevisiae* can yield bioethanol as the main product. On the other hand, the use of this fraction towards the growth of oleaginous yeasts strains like *Metschnikowia pulcherrima* presents the potential to be a source of lipids. Additionally, such substrate can also be used in the growth of microalgae species that can then serve as a feedstock in a biorefinery. Such approach would fit in the framework of integrated biorefineries.

In the second research chapter, SCG was blended and co-processed with microalgae. The results obtained with these blends were satisfactory in the biorefinery configuration used. In fact, the blends demonstrated higher production bioethanol than the microalgae when processed alone. However, the lipids extracted from microalgae were higher than the ones obtained with the blends. Even though, this can be explained by the higher content of lipids in microalgae than in SCG, which consequently leads to lower lipid

content in the blends. Therefore, an improvement to this study could include the optimisation of the lipid extraction yields in SCG. In fact, the lipid extraction yields on SCG were considerably lower than the ones observed in microalgae and the blends. This is caused by the formation of an emulsion between the SCG fermented broth and the extraction solvent, hexane. This emulsion can be broken by increasing the extraction agitation or increasing the extraction temperature. Alternatively, the yields obtained can be increased by adding further extraction steps.

The third research chapter evaluated the use of blends of macroalgae and SCG in an integrated biorefinery approach towards the production of HMF. These blends demonstrated higher production of HMF than the microalgae when processed alone proving that the combination of these feedstocks provide ideal conditions towards the production of HMF. The same experiment was performed with defatted feedstock. This yielded considerably lower concentrations of HMF, which suggests that the lipid layer has an important role towards the production of HMF. An alternative use of these feedstock could be in the production of bioethanol through fermentation due to their large content in carbohydrates. However, the HMF content after the acid dehydration acts as an inhibitor to the microorganisms. Therefore, a lipid extraction is suggested previous to the acid dehydration process. This would reduce the production of HMF in the acid dehydration, allowing for the use of microorganisms to produce products such as ethanol or butanol.

The experimental studies in this thesis have considered the feasibility of using SCG as a feedstock or as a blending agent in integrated biorefineries. However, to make SCG a feasible feedstock in this context, further assessment is required. The seasonality of SCG, collection points, quantities available for collection and costs of transportation are some of the aspects to take into consideration when planning an upstream supply chain.

The seasonality does not seem to be an issue as the coffee is consumed all year round and the production of SCG follows this trend, making this feedstock available through the entire year. Such seasonal behaviour has led to an increasing number of coffee shops worldwide, mainly in large metropolitan areas. Therefore, a transportation and collection supply chain is required. Trucks seem to be the most reasonable transportation method to be used in such supply chain. This is due to the very scattered locations of coffee shops in city centres. However, no studies have been done in this area and there is no data on how this supply chain would look like and how much these transportation costs would be.

Other important decision is on centralised SCG-based biorefinery or several and smaller processing units scattered across the countries. Previous studies have demonstrated that to make the biorefinery profitable, a 42 000 tonne/y biorefinery would be necessary. Considering such number, centralised processing must be considered the most valid option, as this would lead to a suitable economy of scale. It has also been demonstrated that the production/recovery of maximum number of products would influence on the profitability of the facility. This not only includes the production of bioethanol and biodiesel, but other SCG components such as chlorogenic acid and/or lignin and its monomers. Therefore, further research is needed around SCG-based biorefineries to extract further value-added products from SCG.

In this thesis the lab scale co-production of microalgae, macroalgae and spent coffee grounds has been established. The next stage would therefore be to model the potential process performing a techno-economic analysis of the biorefinery designs presented in this thesis. This would help to understand if these biorefineries can be profitable and a reliable source of biofuels in the near future. Further process integration could also be developed including energy integration and PINCH analysis.

If this is successful, then following on the concept developed on this work that blends of different types of biomass can be used in biorefineries to mitigate seasonality problems, further experimental work is proposed on this field. Additionally to blending of SCG with microalgae and macroalgae, SCG can also be tested with other types of biomass. This could lead to improved conditions towards the production of certain products as it happened in the case of HMF.

Additional work can also be done on studying the seasonality of the numerous macroalgae species and combining those with higher production/availability in summer, for example, with the species with lower production/availability in the same season. This way, when one of the species has a lower availability, the other can make up for such low availability and vice-versa. With such an approach, a reliable biorefinery can be build based on this feedstock.

Finally, further municipal wastes should be tested and blended with seasonal biomass sources. This could include wastes such as the wastes produced in gardening or food wastes like fruit and vegetable peels.